

TECHNICAL REPORT

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Thermoregulatory and Immune Responses During Cold Exposure: Effects of
Repeated Cold Exposure and Acute Exercise

by

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CONTENTS

TABLES.....	v
FIGURES.....	vi
EXECUTIVE SUMMARY.....	1
BACKGROUND.....	2
PHASE ONE.....	4
REPEATED IMMERSIONS	4
INTRODUCTION.....	4
METHODS.....	5
RESULTS.....	9
DISCUSSION	19
TIME OF DAY EFFECTS.....	24
INTRODUCTION.....	24
METHODS.....	25
RESULTS.....	28
DISCUSSION	32
PHASE TWO.....	35
PRIOR EXERCISE	35
INTRODUCTION.....	35
METHODS.....	37
RESULTS.....	40
DISCUSSION	46
IMMUNE RESPONES	51

INTRODUCTION	51
METHODS.....	52
RESULTS	60
DISCUSSION	72
REFERENCES	82

TABLES

Table 1. Intercept and slope values for \bar{T}_b - $\Delta\dot{M}$ relationship.....14

Table 2. Multiple regression analysis for prediction of leukocyte subset and lymphocyte counts by heart rate, rectal temperature and hormone concentrations.....66

FIGURES

Fig. 1. Rectal temperature vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$	10
Fig. 2. Metabolic heat production vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$. # Main effect, Repeat significantly lower than Control, $P < 0.05$	12
Fig. 3. Cumulative heat storage vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$	13
Fig. 4. Plasma norepinephrine vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$	17
Fig. 5. Thermal sensation vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. #, Main effect, Repeat significantly higher than Control, $P < 0.05$	18
Fig. 6. Rectal temperature and change in rectal temperature vs. time for 0700 and 1500 during cold water immersion. *, Significant difference between 0700 and 1500, $P < 0.05$	29

Fig. 7. Mean skin temperature and rectal-skin gradient vs. time for 0700 and 1500 during cold water immersion. *, Significant difference between 0700 and 1500, $P < 0.05$.	30
Fig. 8. Heat flow and metabolic heat production vs. time for 0700 and 1500 experiments during cold water immersion.	31
Fig. 9. Rectal temperature vs. time for exercise and passive heating experiments during cold air exposure. Postimmersion, temperature at end of water immersion. *, Exercise significantly different ($P < 0.05$) than Control at specified times.	42
Fig. 10. Mean skin temperature (A) and rectal-skin temperature gradient (B) vs. time for exercise and passive heating experiments during cold air exposure.	43
Fig. 11. Mean weighted heat flow (A) and insulation (B) vs. time for exercise and passive heating experiments during cold air exposure. #, Main effect, EX significantly different from Control ($P < 0.05$).	44
Fig 12. Individual heat flow (A) and insulation (B) for 8 sites measured. *, calf heat flow was higher ($P < 0.05$) and insulation lower ($P < 0.05$) during EX.	45
Fig. 13. Rectal temperature during water exposure and cold air exposure.	62
Fig. 14. Leukocyte, granulocyte, lymphocyte, and monocyte counts during water exposure and cold air exposure. *, significant difference ($P < 0.05$) from initial measurement (pre vs. post, time 0 vs. time 60 & 120). a, Sit-35°C different than EX-18°C; b, Sit-35°C different than EX-35°C; c, Sit-38°C different than EX-35°C; d, EX-35°C different than EX-18°C at corresponding time.	63

Fig. 15. Natural killer cell counts, total natural killer cell activity (lytic units) and lytic units per number of natural killer cell during water exposure and cold air exposure. *, significant difference ($P < 0.05$) from initial measurement (pre vs. post, time 0 vs. time 60 & 120). a, Sit-35°C different than EX-18°C; c, Sit-38°C different than EX-35°C.	67
Fig. 16. Plasma norepinephrine (A), epinephrine (B), and cortisol (C) concentrations during cold air exposure following each treatment.	70
Fig. 17. Interleukin-6 concentrations during water exposure (A) and cold air exposure (B) during each treatment. c, Sit-38°C different ($P < 0.05$) than EX-35°C; d, EX-35°C different ($P < 0.05$) than EX-18°C at corresponding time.	71

EXECUTIVE SUMMARY

Whether or not the thermoregulatory system fatigues (i.e., causes blunted shivering thermogenesis and vasoconstriction) during cold exposure is unknown. Thus, a series of experiments were executed to determine if serial cold water immersion or acute exertional fatigue would cause thermoregulatory fatigue during subsequent cold exposure. This information will be used in developing thermoregulatory models during cold exposure. During these studies several unanswered questions regarding thermoregulation in the cold were also addressed: 1) do thermoregulatory responses to cold show fatigue when prolonged cold water exposures are serially repeated? 2) are thermoregulatory effector responses to cold affected by time of day? (i.e., circadian influences) 3) does a single acute, fatiguing exercise bout impair thermoregulatory responses to subsequent cold exposure? and 4) does fatigue induced by severely strenuous physical activity compromise the immune response to cold exposure? The results of these studies suggest that 1) serial cold water blunts shivering leading to lower core temperatures, 2) thermoregulatory effector responses are not affected by time of day, 3) exercise increases peripheral heat loss and lowers core temperature during subsequent cold exposure, and 4) that acute cold exposure has immununostimulating effects.

BACKGROUND

Case studies and anecdotal reports suggest that fatigued individuals have an increased susceptibility to hypothermia in cold environments. However, reports outlining the effects of fatigue on thermoregulatory responses to cold water or air are few (78,79). In February 1995, four students at the U.S. Army Ranger School died of hypothermia during training in the swamp. The fact that this occurred at the end of a 9 week training course which, by design, requires students to endure chronic physical exhaustion rekindled interest in fatigue as a hypothermia predisposition factor.

Following this incident, USARIEM performed a study (115) of Ranger School students immediately following training documenting that individuals who experienced the multi-stressor environment of Ranger School (i.e., chronic exertional fatigue, sleep loss, underfeeding) exhibited more rapid body cooling due to reduced tissue insulation and a blunted metabolic heat production response to cold exposure, compared to responses to cold measured after recovery from the school. These findings suggested that such a multi-stressor environment might induce "thermoregulatory fatigue", i.e. cause a blunting of shivering thermogenesis and peripheral vasoconstriction. To address this possibility, we developed and executed a comprehensive series of controlled studies to examine the effect of various factors on thermoregulation in the cold. A systematic approach was utilized to elucidate the mechanisms by which fatigue may impair thermoregulatory responses during cold exposure. Also, we examined the effects of fatigue on immune function during cold exposure. The experiments described in this technical report were conducted in two phases. Each phase answered a set of questions and the two phases were not dependent on each other. The experimental studies addressed the following

specific questions: 1) do thermoregulatory responses to cold become fatigued when prolonged cold water exposures are serially repeated? 2) are thermoregulatory effector responses affected by time of day 3) does a single bout of acute, fatiguing exercise impair thermoregulatory responses to subsequent cold exposure? and 4) does acute fatigue compromise the immune response to cold exposure?

PHASE ONE

REPEATED IMMERSIONS

INTRODUCTION

Soldiers, sportsmen, emergency rescue teams, and others may remain outdoors for extended periods during cold weather often combined with rain, snow and wind. Human thermoregulatory effector responses to cold act in concert to maintain normothermia and include shivering thermogenesis, which increases metabolic heat production, and peripheral vasoconstriction, which decreases body heat loss. If these adjustments are inadequate to maintain the balance between heat production and heat loss, which is often the case in cold-wet conditions due to the high thermal conductivity of water, a heat debt develops, manifested by a fall in core temperature.

Some evidence suggests that the thermoregulatory system might fatigue during prolonged cold exposures. Pugh (78,79) provided anecdotal evidence that fatigue of shivering may have occurred in people hiking in cold-wet conditions. More quantitative evidence for shivering fatigue has been reported by Bell et al. (4) and, most recently, by Thompson and Hayward (105). Recent work has also demonstrated that smooth muscle may also exhibit characteristics of fatigue (113) and if this occurred in vascular smooth muscle, vasoconstrictor responses could be compromised. Possible mechanisms for fatigue of shivering or vasoconstriction fatigue include: 1) depletion of muscle energy substrates fueling shivering metabolism and the central nervous system (CNS), 2) non-metabolic peripheral muscle fatigue, i.e, contractile mechanism failure,

and 3) central (CNS) or peripheral (neuromuscular junction) fatigue of muscle recruitment for shivering/vasoconstriction. No study has specifically attempted to document in a systematic manner whether thermoregulatory fatigue develops during serial cold exposures.

This study examined whether cold water immersions (eliciting decreases in body core temperature) repeated several times in one day would lead to thermoregulatory fatigue. It was hypothesized that during the second and third serial immersions, blunted shivering thermogenesis and reduced peripheral vasoconstriction would lead to a greater fall in core temperature.

METHODS

Subjects. Eight healthy men participated in this study. Physical characteristics were age, 24.4 ± 1.1 (SE) yr; height, 177.8 ± 3.0 cm; mass, 78.8 ± 3.1 kg; body surface area, 1.96 ± 0.05 m²; peak oxygen uptake ($\dot{V}O_{2\text{peak}}$), 49.5 ± 1.6 ml·kg⁻¹·min⁻¹; percent body fat $14.2 \pm 1.3\%$; and skinfold thickness, 2.9 ± 0.5 mm.

Preliminary testing. Body density from underwater weighing corrected for residual lung volume was measured and percent fat calculated according to Siri (94). Mean skinfold thickness was calculated from 10 sites according to Allen et al. (2). All subjects completed an incremental cycle ergometer test to exhaustion for determination of $\dot{V}O_{2\text{peak}}$. Briefly, subjects pedaled at 60 watts for 2 min and the resistance was increased 30 watts every 2 min until exhaustion was achieved.

Experimental design. Subjects reported to the laboratory one hour before their immersion. Subjects refrained from alcohol, tobacco products, medications, and

exercise for 12 hours before all testing. Following instrumentation, subjects were seated for 15 minutes on a platform suspended above the water to obtain pre-immersion measurements. Following these baseline measurements, subjects were quickly lowered into stirred water (20°C) to shoulder level. On 3 separate days, designated as controls, subjects were immersed for 120 min beginning ~ at 0700, 1100, and 1500. These control trials (CONTROL) were separated by at least one week and the order was randomized. Following completion of the 3 control trials, subjects were then immersed 3 times (REPEAT) during the course of one day, with the beginning of each immersion corresponding to the starting time (0700, 1100, 1500) of the three control trials. The duration of these immersions was 120 min. Pre-immersion rectal temperatures (T_{re}) at 1100 and 1500 during REPEAT were matched as closely as possible to the T_{re} recorded at these times during the control experiments. To achieve this match, subjects were passively rewarmed (semi-recumbent, clothed in sweatsuit and covered by one wool blanket) for one hour after the 0700 and 1100 immersions. During min 10-30 of the passive rewarming period following both the 0700 and 1100 immersions, subjects received 3.57 ml·kg⁻¹ of hot chocolate and 16 oz of a commercial liquid food supplement (Ensure®, Ross Laboratories, Columbus, OH). Following the one hour passive rewarming, subjects were placed in a warm shower until their T_{re} matched their respective control 1100 and 1500 pre-immersion T_{re} . Before re-immersion, subjects were seated on the platform above the water for 15 min to obtain baseline body temperature measurements.

Measurements. A thermistor inserted 10 cm past the anal sphincter measured rectal temperature. Mean weighted skin temperature (\bar{T}_{sk} , °C) and mean weighted heat

flow (\bar{H}_c , $W \cdot m^{-2}$) were calculated from T_{sk} and heat flow measurements obtained using an integrated disk system (Concept Engineering heat flow sensor with integral linear thermistor, Old Saybrook, CT) placed at nine skin surface sites. Mean weighted skin temperature ($^{\circ}C$) was calculated (106) as follows: $\bar{T}_{sk} = 0.06T_{foot} + 0.17T_{calf} + 0.14T_{medial thigh} + 0.14T_{lateral thigh} + 0.14T_{chest} + 0.07T_{tricep} + 0.07T_{forearm} + 0.14T_{subscapular} + 0.07T_{hand}$. Mean weighted heat flow ($W \cdot m^{-2}$) was calculated (21) as follows: $\bar{H}_c = 0.28H_{subscapular} + 0.14H_{forearm} + 0.08H_{triceps} + 0.22H_{calf} + 0.28H_{thigh}$. Mean body temperature (\bar{T}_b) was calculated (37) as follows: pre-immersion, $\bar{T}_b = 0.8T_{re} + 0.2 \bar{T}_{sk}$; during immersion, $\bar{T}_b = 0.67T_{re} + 0.33 \bar{T}_{sk}$. Tissue insulation was measured (37) as follows: $I_T = (T_{re} - \bar{T}_{sk}) / \bar{H}_c$. Individual skin conductance at the site of each heat flow disk was calculated as the reciprocal of I_T . Temperature and heat flow measurements were continuously recorded using a computer-automated data acquisition system.

Oxygen uptake (O_2) was measured via open circuit spirometry using an automated metabolic analysis system (Model 2900, SensorMedics, Yorba Linda, CA). Measurements were obtained during preimmersion and at minutes 5-15, 25-35, 45-55, 65-75, 85-95, and 105-115 of immersion. Metabolic heat production (\dot{M} , $W \cdot m^{-2}$) was estimated from the O_2 and respiratory exchange ratio (R) using the following equation (37): $\dot{M} = (0.23[R] + 0.77) \cdot (5.873)(\dot{V}O_2) \cdot (60/A_D)$, where A_D is body surface area (m^2), derived from the DuBois and DuBois equation (30).

Body heat storage (\dot{S} , $W \cdot m^{-2}$) was calculated as follows (37): $\pm \dot{S} = \dot{M} - \dot{W} - \dot{L} - \dot{E} - \dot{K} - (\dot{R} + \dot{C})$, where \dot{M} is the metabolic rate, \dot{W} is work rate (0 in this experiment), \dot{L} is the respiratory heat losses by convection and evaporation (22), \dot{E} is evaporative heat loss (set at $4.1 W \cdot m^{-2}$ in this experiment) (22), \dot{K} represents conductive heat loss (0 in this

experiment) and $\dot{R} + \dot{C}$ represents dry heat loss. Cumulative body heat debt was expressed as a positive number and was defined as the total negative heat storage integrated over time. Thermal sensation (TS) was rated using a category rating scale (117) at min 0, 15, 35, 55, 75, 95, and 115 of exposure.

Blood. Whole blood samples were drawn at pre-immersion (min 0) and at minutes 30, 60, 90, and 120 of immersion from an indwelling venous catheter (18 gauge) placed in a superficial forearm vein. Aliquots were centrifuged at 4°C to separate the plasma. Plasma samples were frozen at -40°C before analysis. Plasma glucose concentration was determined in duplicate by auto-analyzer (Model 2300, Yellow Springs Instrument Inc., Yellow Springs, OH). Plasma norepinephrine (NE) concentration was determined (48) in duplicate via high performance liquid chromatography with electrochemical detection (Model 460, Waters, Inc.).

Statistical Analyses. A two-way repeated measures analysis of variance was utilized to determine if significant differences existed between the appropriate control (0700, 1100, 1500) condition and the REPEAT trial at the same time of day. Significant F-ratios were analyzed post-hoc using Newman-Keuls tests. The slope and intercept of each individual's \bar{T}_b vs change in \dot{M} ($\Delta\dot{M}$) relationship during immersion was determined by least squares linear regression. Paired t-tests were used to determine if differences in slope or intercept data existed between CONTROL and REPEAT for \bar{T}_b vs. $\Delta\dot{M}$. Data are reported as means \pm S.E. Significance was determined at $p < 0.05$.

RESULTS

Rectal temperature. Rectal temperatures (T_{re}) before and during the immersions are depicted in Figure 1. During the 0700 immersion, there were no significant differences in T_{re} across time between CONTROL and REPEAT. However, during the 1100 immersion, T_{re} was significantly lower at min 120 of the REPEAT trial and there was a tendency ($p < 0.12$) for a similar effect in REPEAT during the last 20 min of the 1500 immersion. The cooling rate ($^{\circ}\text{C}\cdot\text{hr}^{-1}$) from min 60 to min 120 of CONTROL and REPEAT immersions, respectively, was: 0700, -0.62 ± 0.1 and -0.65 ± 0.1 ; 1100 ($p = 0.21$), -0.55 ± 0.1 and -0.74 ± 0.1 ; 1500 ($p = 0.13$), -0.55 ± 0.1 and -0.73 ± 0.1 .

Skin temperature. There were no significant differences in mean skin temperature (\bar{T}_{sk}) between trials at 0700, 1100, or 1500, either before or during the cold water immersion.

Heat flow. Pre-immersion \bar{H}_c ($\text{W}\cdot\text{m}^{-2}$) was significantly higher in REPEAT vs. CONTROL before the 1100 (128 ± 7 vs. 71 ± 3) and 1500 (120 ± 9 vs. 71 ± 3) immersions. However, after subjects were immersed, there were no significant differences in \bar{H}_c between trials. \bar{H}_c increased to approximately $500 \text{ W}\cdot\text{m}^{-2}$ at min 5 of immersion, then fell to approximately $250 \pm 20 \text{ W}\cdot\text{m}^{-2}$ at 60 min of immersion. Final I_T ($^{\circ}\text{C}\cdot\text{m}^2\cdot\text{W}^{-1}$) were not different between trials at 0700 (0.06 ± 0.01 vs 0.06 ± 0.01), 1100 (0.06 ± 0.01 vs. 0.07 ± 0.01), and 1500 (0.06 ± 0.01 vs. 0.07 ± 0.01) for CONTROL and REPEAT, respectively. Skin conductance was not significantly different at 1100 between trials at any of the 9 individual sites measured.

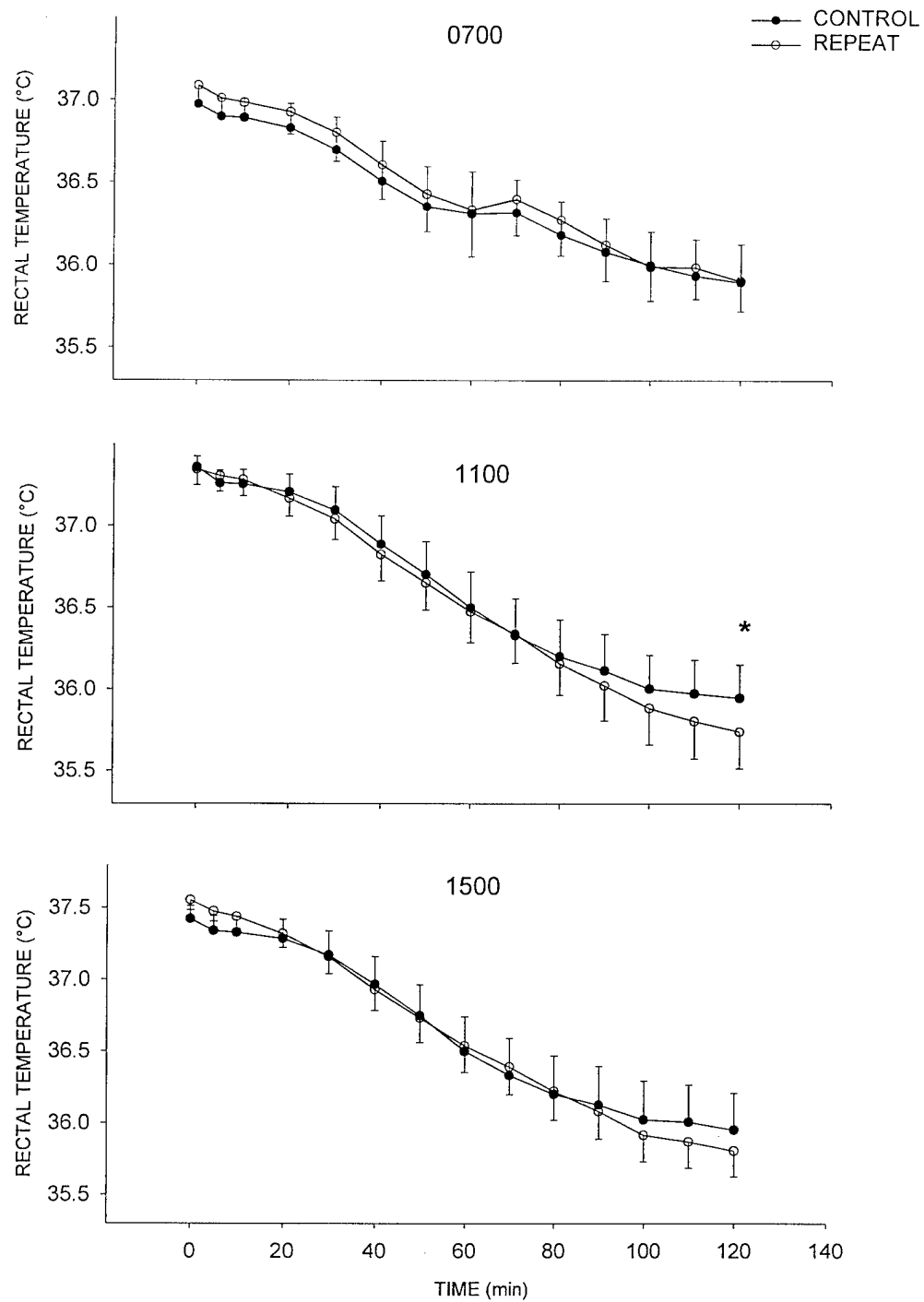


Fig. 1. Rectal temperature vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$

Metabolic heat production. Pre-immersion did not differ between trials at 0700, 1100, or 1500 immersions (Figure 2). \dot{M} increased approximately 2.5-3 fold after subjects were immersed and they appeared to be shivering vigorously. During the 0700 immersion, there were no significant differences in \dot{M} except at min 75, when \dot{M} was lower ($p < 0.05$) during REPEAT (Figure 2). During the 1100 and 1500 immersions, \dot{M} was significantly ($p < 0.05$) lower in REPEAT vs. CONTROL.

Heat Debt. Heat debt across time was similar between trials at 0700 and 1500 (Figure 3). However, heat debt was significantly higher in REPEAT at min 115 during the 1100 immersion trial.

\bar{T}_b vs. $\Delta\dot{M}$ relationship. The relationships between mean body temperature (\bar{T}_b) and the corresponding increment in metabolic heat production over pre-immersion values ($\Delta\dot{M}$, a measure of shivering thermogenesis) are presented in Table 1. During the 0700 immersion, there were no differences between trials in either the intercept or the slope of the \bar{T}_b vs. $\Delta\dot{M}$ relationship. During the 1100 immersion, the intercept for \bar{T}_b was significantly lower in REPEAT than CONTROL, but slopes were not different between trials. During the 1500 immersion, there were no differences between trials in either the intercept or slope of the \bar{T}_b vs. $\Delta\dot{M}$ relationship.

Plasma Glucose. Pre-immersion glucose concentrations ($\text{mmol}\cdot\text{l}^{-1}$) were: 4.7 ± 0.2 and 5.1 ± 0.1 at 0700; 5.1 ± 0.2 and 5.8 ± 0.3 at 1100; and 5.1 ± 0.5 and 6.6 ± 0.6 at 1500, for CONTROL and REPEAT, respectively. Glucose concentrations at min 120 were: 5.0 ± 0.2 and 5.0 ± 0.1 at 0700; 4.9 ± 0.2 and 4.8 ± 0.1 at 1100; and 4.9 ± 0.1 and 4.8 ± 0.1 at 1500, for CONTROL and REPEAT, respectively.

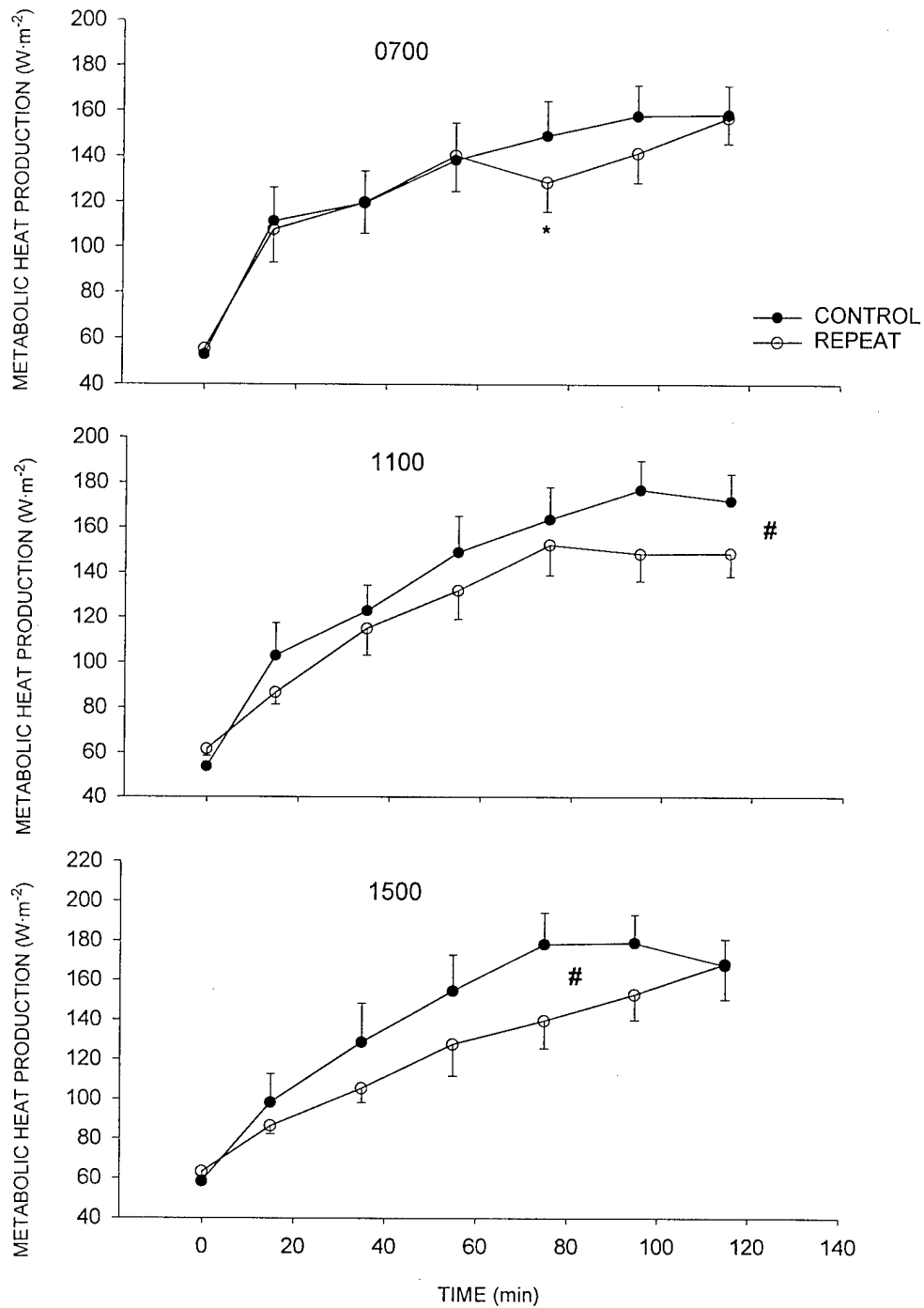


Fig. 2. Metabolic heat production vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$. #, Main effect, Repeat significantly lower than Control, $P < 0.05$.

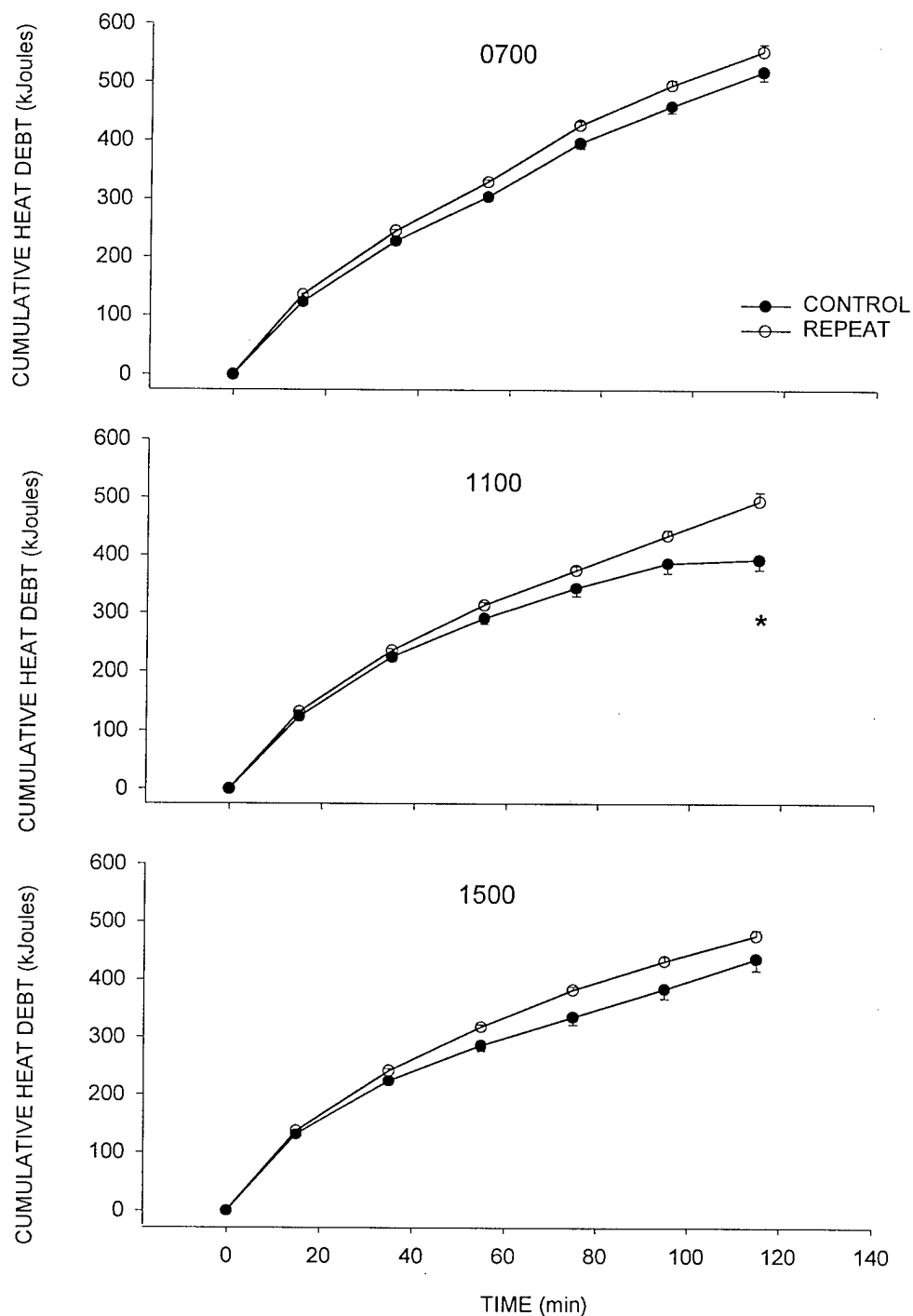


Fig. 3. Cumulative heat storage vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$.

Table 1. Intercept and slope values for T_b - ΔM relationship.

S #	Intercept					
	700		1100		1500	
	Control	Repeat	Control	Repeat	Control	Repeat
1	33.16	33.72	33.15	33.2	33.45	32.76
2	32.82	32.99	34.12	33.12	32.95	33.02
3	32.13	32.25	32.37	32.3	32.22	32.58
4	32.79	32.29	32.27	32.42	32.61	32.34
5	33.8	33.13	34.12	32.81	34.35	33.53
6	32.76	32.69	33.68	32.53		
7	32.63	32.39	33.09	32.49	32.53	32.11
8	31.86	32.01	32.57	32.3	32.28	32.45
Mean	32.74	32.68	33.17	32.64 *	32.91	32.68
S.E.	0.21	0.2	0.26	0.13	0.29	0.18

S #	Slope			
	700		1500	
	Control	Repeat	Control	Repeat
1	-64.95	-49.91	-66.8	-62.29
2	-78.83	-52.85	-36.31	-39.4
3	-49.39	-49.32	-53.49	-36.28
4	-45.4	-109.96	-104.23	-105.13
5	-59.37	-75.82	-60.14	-79.27
6	-61.63	-60.8	-46.24	-46.93
7	-80.26	-78	-60.18	-70.21
8	-71.91	-76.31	-58.31	-53.47
Mean	-63.97	-69.12	-60.71	-61.62
S.E.	4.5	7.24	7.06	8.12
				5.9
				8.62

T_b, mean body temperature; ΔM, change in metabolic heat production; *, Repeat significantly different than Control

Plasma Norepinephrine. Figure 4 depicts the plasma NE concentrations during all three immersions. There were no differences observed at 0700. However, at 1100 and 1500, plasma NE was significantly higher before immersion in REPEAT vs. CONTROL. NE concentrations during immersion did not differ between trials.

Thermal Sensation. During 0700 or 1500 immersions (Figure 5), there were no differences in TS between trials. However, during 1100 immersion, TS was significantly higher in REPEAT vs. CONTROL, (i.e. subjects perceived the immersion as warmer).

Amnesia. An interesting finding study from this study was the occurrence of amnesia in one subject. The subject reported no symptoms during the first two immersions (0700 and 1100) during the REPEAT day. During REPEAT at 1100, T_{re} was approximately 0.3°C lower after 2 hours of immersion. During the 1500 REPEAT immersion, the subject reported feeling exhausted, tired, and that his muscles were sore. However, nothing remarkable occurred until min 85 of immersion. At this point, the principal investigator observed that the volunteer turned pale. At min 90 the medical monitor was called and a technician stationed in the water next to the volunteer. The subject responded to questions from the medical monitor from min 95 to min 115, at which point his face fell into the water and the experiment was stopped. During this 20 min, the volunteer exhibited altered affect (i.e. whimpering, anxious delirium-like state), yet remained alert, oriented, and repeatedly insisted that he be allowed to complete the experiment. Oxygen uptake ($\dot{V}O_2$) was measured from min 105-112. After stopping the test, the subject was stabilized on a poolside platform for 7 min. He then walked down several stairs before assuming a semi-recumbent position for rewarming. At this point, the investigator recounted to the volunteer the symptoms that the volunteer exhibited.

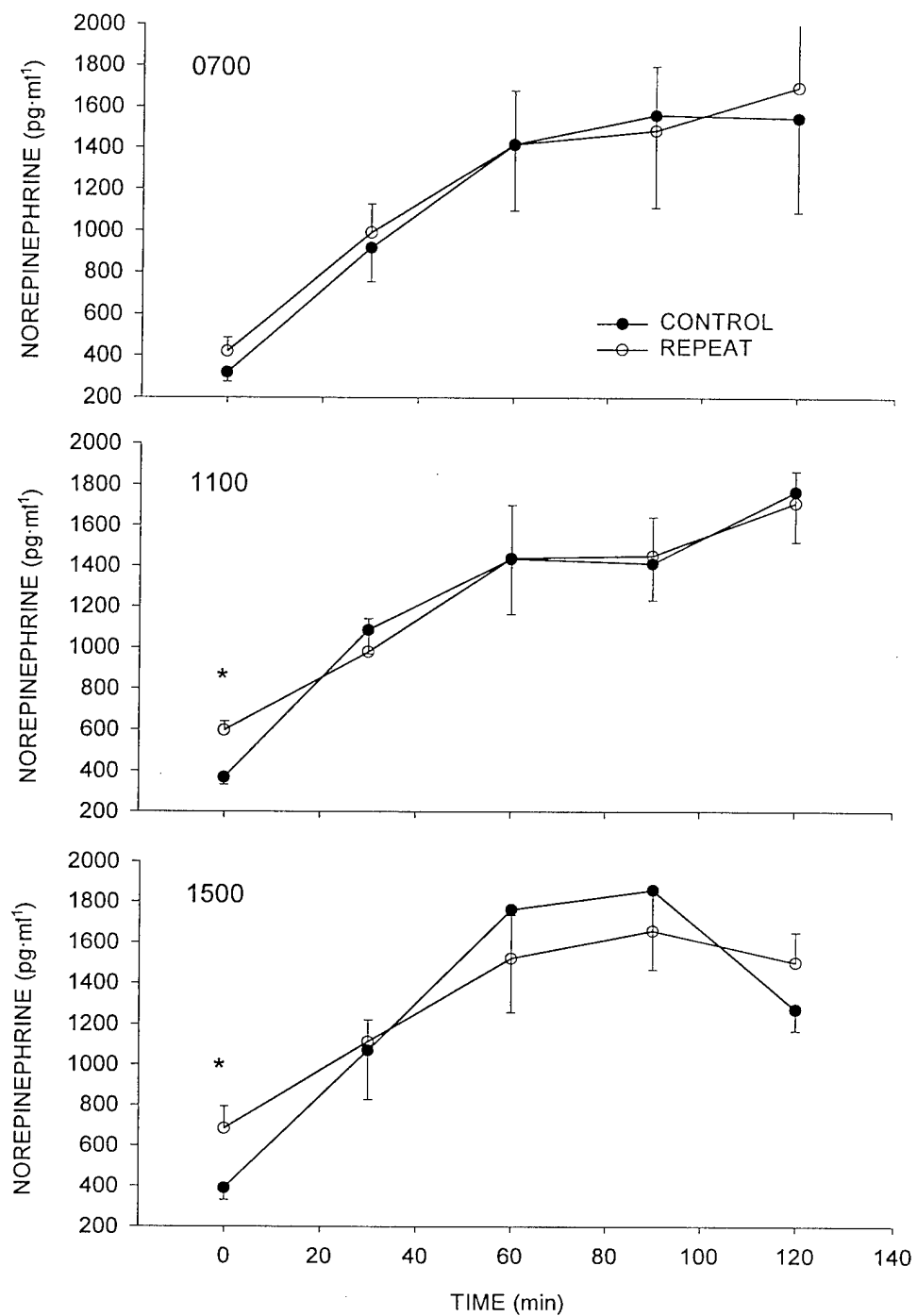


Fig. 4. Plasma norepinephrine vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. *, Repeat significantly different from Control, $P < 0.05$.

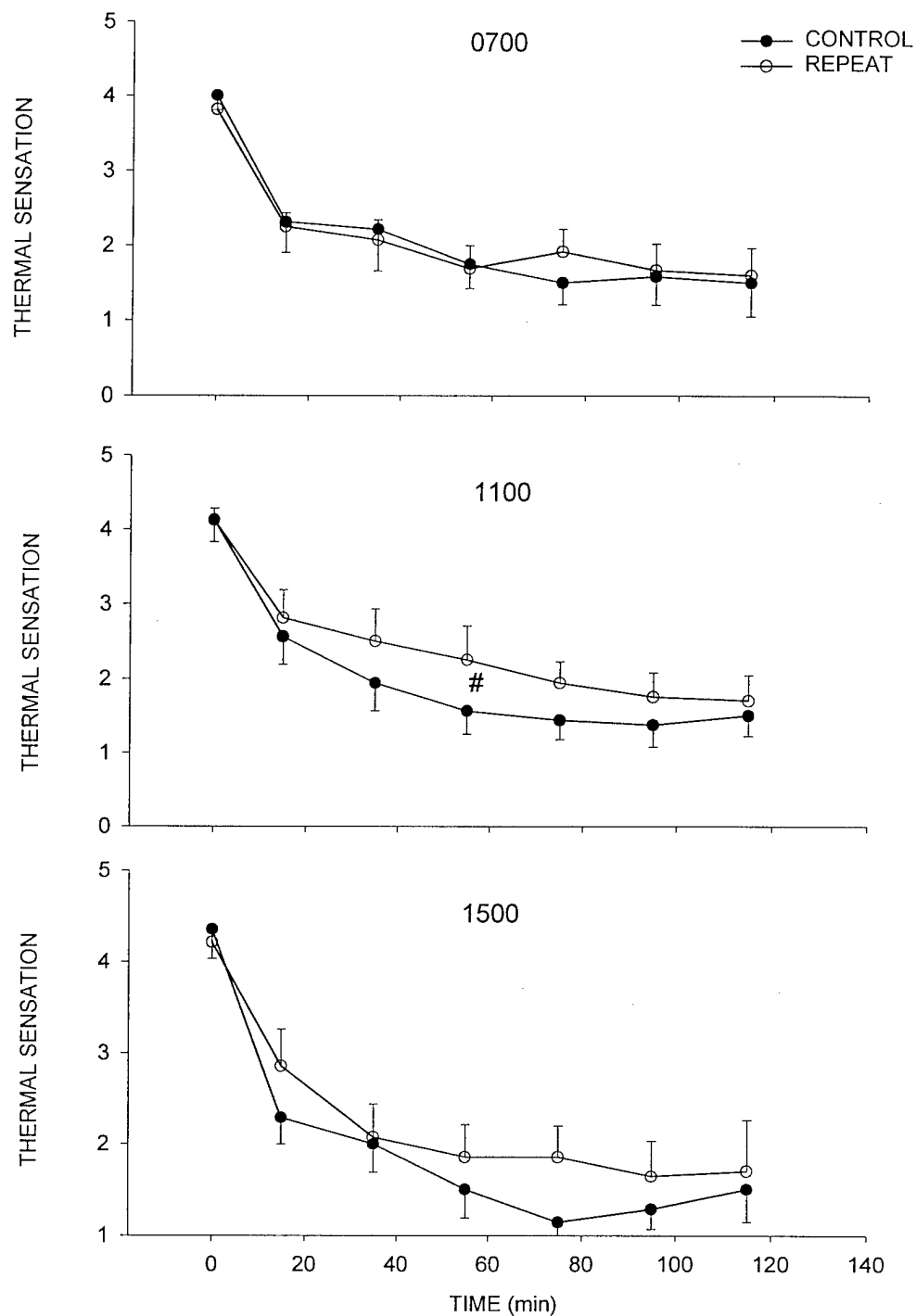


Fig. 5. Thermal sensation vs. time at 0700, 1100, and 1500 for serial and single experiments during cold water (20°C) immersion. #, Main effect, Repeat significantly higher than Control, $P < 0.05$.

The volunteer did not recall these symptoms, nor did he recall the $\dot{V}O_2$ measurement, which requires the volunteer to place and breathe through a mouthpiece. Further questioning by the medical monitor revealed that the last clearly recalled event by the subject occurred at min 95 of immersion. This lack of memory recall lasted until min 115 when the subject was raised out of the water. During REPEAT trial at 1500, T_{re} was 35.6°C at min 95 and rose 0.1°C over the next 20 min when the test was terminated. In comparison, during CONTROL, T_{re} at min 95 was 35.5°C and remained at 35.5°C through min 115. Plasma glucose concentrations at min 90 were 4.72 and 4.97 mmol·l⁻¹, for CONTROL and REPEAT respectively. Plasma norepinephrine concentrations were 14.72 and 14.80 nmol·l⁻¹, for CONTROL and REPEAT respectively.

DISCUSSION

This study investigated whether thermal balance and thermoregulatory responses during cold water immersion would be degraded over the course of several serial immersions completed in a single day. The hypothesis was that the thermoregulatory system could become “fatigued” and unable to maintain thermal balance as effectively during subsequent exposures. No previous studies have systematically evaluated repeated cold exposure in one day controlling for factors such as circadian rhythm and initial core temperature.

The principal finding of this study was that T_{re} was significantly lower in REPEAT, vs. CONTROL, at the end of the 1100 immersion. There was also a greater heat debt in the REPEAT vs. CONTROL trial during the 1100 immersion. Even larger differences in T_{re} might have been observed if the immersion had continued beyond two hours due

to the $0.2^{\circ}\text{C}\cdot\text{hr}^{-1}$ difference in cooling rate that had developed by this point. The T_{re} and heat debt data from the 1500 trial was also consistent with this pattern, although differences between CONTROL and REPEAT did not achieve statistical significance. These observations tend to support the hypothesis, and suggest that susceptibility to hypothermia may increase with repeated cold exposures completed during a single day.

The lower T_{re} observed by the end of immersion in the 1100 REPEAT trial appears to be due to an attenuated thermogenic response to cold and not a loss of vasoconstriction. No differences between trials in \bar{H}_c or I_T suggests that peripheral heat loss was not affected by multiple cold exposures and suggests that there was no fatigue of cutaneous vascular smooth muscle or vasoconstrictor neural drive. A blunted thermogenic response was supported by metabolic heat production and \bar{T}_b vs. $\Delta\dot{M}$ data. The decrease in metabolic heat production may also partially result from a delay in the onset of shivering. The intercept for the \bar{T}_b vs. $\Delta\dot{M}$ relationship shifted such that the increase in \dot{M} during the 1100 REPEAT exposure was not observed until the subjects achieved a lower \bar{T}_b . Therefore, at any given \bar{T}_b , \dot{M} was lower in the REPEAT trial vs. CONTROL at 1100.

A similar intercept shift in the shivering response has also been demonstrated after N_2 narcosis (69), anesthesia (86), hypercapnia (55), hypoglycemia (74), and an increase in T_{sk} (8,20). A leftward shift in the intercept of the \bar{T}_b vs. $\Delta\dot{M}$ relationship has been interpreted as an alteration in the central reference mechanism controlling thermoregulatory effector responses (100). The subjects in the present experiments were breathing air at sea level barometric pressure, so anesthetic effects or nitrogen levels in the body do not explain the shivering suppression, nor is it likely that they were

hypercapnic. Plasma glucose concentrations were well above $2.5 \text{ mmol}\cdot\text{l}^{-1}$ during the course of any immersion and \bar{T}_{sk} were similar between trials during all three immersion periods.

Another possible explanation for the apparent blunting of \dot{M} observed with serial immersion is that it represents the early development of shivering habituation. In previous studies involving cold exposures, shivering habituation has developed over the course of a number of days or weeks of repeated cold exposure (12,47,114). The interesting possibility arises that even as few as one or two cold exposures may be a sufficient stimulus for adaptation to cold to begin. The blunting of thermal sensation also suggests habituation. However, in previous investigations, cold habituation blunted the vasoconstrictor and sympathetic responses to cold (64,80). In the present study, no blunting of plasma NE response to cold was seen, nor were there any apparent changes in the vasomotor response to cold. Studies demonstrating habituation of vasomotor and sympathetic responses to cold, however, employed cold air exposures in contrast to the cold water immersions used in the present experiments. Immersion may mask any changes in T_{sk} due to the near matching of T_{water} to T_{sk} . Further, the large rates of heat loss may elicit maximal sympathetic responses in both acclimated and unacclimated persons. Thus, even if a relatively small number of cold water exposures are sufficient to begin the development of metabolic and perceptual adaptations to cold, more exposures are needed for thermoregulatory effector responses to fully develop.

The effect of circadian rhythms on responses to cold has not been explored. Potentially due to a circadian rhythm effect, during the 1100 CONTROL, there was a

tendency for the mean intercept, compared to the other controls, to be higher ($p = 0.18$). And, although the mean intercept during 1100 REPEAT was similar to 0700 and 1500 REPEAT, this value may, in fact, reflect a blunting of the normal circadian response at 1100 due to multiple immersions. The slope of \bar{T}_b vs. $\dot{\Delta M}$ was not altered, suggesting that the sensitivity of the metabolic response to a given change in body core temperature was unchanged. Further studies examining time of day effects on thermoregulatory responses to cold are needed.

The hypothesis of this experiment was that serial cold water immersions, repeated over a short period, would lead to an inability to thermoregulate effectively, thus increasing a person's risk of hypothermia. Our data suggest that this in fact may be the case, as individuals were unable to maintain body temperature as well after being cold exposed before a subsequent cold exposure. It appears that this reduction is due to an attenuation of the metabolic heat response to the cold. However, these results may also be explained by the early development of cold habituation. Further studies are needed to determine if the thermoregulatory system "fatigues" with repeated cold exposures, but the possibility that cold adaptation can develop more rapidly than thought must be considered when designing experiments.

Amnesia. Brown and Hachinski (11) define transient global amnesia (TGA) as "a syndrome in which a previously well person suddenly becomes confused and amnesic." The interesting finding is that a person had a lack of memory recall during cold exposure at a time when rectal temperature measurements demonstrated that he was not clinically hypothermic. TGA typically is 20 minutes in duration and is common in older individuals. It is atypical in a young person. The physiology of TGA is also quite

different than hypothermia-induced amnesia. For example, the EEG in TGA shows a different firing pattern than hypothermia-induced amnesia, which is characterized by a decrease in alpha activity (34). Most amnesia reports during cold exposures are anecdotal. However, Keatinge (57) reported amnesia in two subjects cooled to core temperatures of 34.2 and 35.1°C. Impaired memory during cold water immersion has also been demonstrated by Coleshaw et al. (21). Fisher (33) reviewed 78 cases of TGA and found 3 were precipitated by cold water. Other precipitating events include emotional experiences, sexual intercourse, and pain (33).

The underlying physiologic cause of amnesia and delirium in this experiment is not apparent, although cold water, migraines, and other stresses typically precipitate TGA. Potential mechanisms for TGA include vertebral arterial spasm (52), transient ischemic attacks (35), and other changes associated with migraines (107). Follow-up studies on this subject to rule out underlying structural causes, epilepsy, or vascular stenosis were not conducted (11). A potential etiology for this amnesic episode, suggested by the subject's pallor, is a change in cardiovascular function. However, cardiac output and total peripheral resistance are reportedly unchanged during this type of immersion (31).

The experimental treatment (3 immersions and 2 rewarmings over a 10 hour period) may itself have led to the amnesia. Hypoglycemia was ruled as a factor as plasma glucose levels remained normal. Body temperature fluctuations or repeated cold water exposures could lead to endotoxemia or other metabolic disturbances during rewarming. However, there were no gastrointestinal disturbances or fever noted.

Regardless of the physiologic mechanisms, memory loss and confusion induced by cold-exposure without hypothermia is an important observation. This demonstrates how decision making could be impaired in fatigued-cold persons. Clearly, prevention of hypothermia alone is not sufficient to defend persons from this problem. Amnesia occurring at this relatively non-hypothermic body temperature must be a concern for individuals who experience low body temperatures in non-controlled settings such as professional or recreational diving. Also prolonged exposure to cold, rainy weather may increase the risk of mental impairment and behavioral changes (76). Memory lapses and amnesia during cold exposure may affect decision making, thereby leading to more serious complications.

TIME OF DAY EFFECTS

INTRODUCTION

Resting core temperatures (T_{core}) vary throughout the day, according to an intrinsic circadian rhythm. Typically, T_{core} achieves its nadir in the early morning and then rises to a peak in the late afternoon. Thermoregulatory responses to exercise and heat stress (e.g., T_{core} threshold for the initiation of sweating and forearm blood flow) also vary over the day (100,109). These threshold changes closely parallel the change in resting T_{core} . Whether human thermoregulatory responses to cold stress exhibit similar rhythmicity has not been documented. Information regarding a possible circadian pattern to thermoregulatory responses to cold stress has important implications for experimental designs and perhaps predicting susceptibility to cold injury.

This study examined whether shivering thermogenesis or vasoconstriction during cold water immersion differs between morning and afternoon exposure. It was hypothesized that the shivering response to cold exposure would vary with time of day, such that with the circadian rise in T_{core} , the onset of shivering would occur at a higher body temperature. We also hypothesized that the vasomotor responses governing peripheral heat loss during cold exposure would also exhibit a time of day effect, although we could not predict the direction of that effect. Experimental findings reported could support a shift in either direction. On the one hand, plasma norepinephrine has been shown to be higher in the afternoon vs. morning (97), so peripheral heat losses may also be less in the afternoon due to greater sympathetically mediated peripheral vasoconstriction. On the other hand, radiative and convective heat loss (via an increase in resting forearm blood flow) is greater in the afternoon vs. morning (72,99), which would lead to greater heat loss.

METHODS

Subjects. Nine males participated in this study after being fully briefed on the risks and giving informed consent. Physical characteristics were: age, 23.8 ± 1.1 (SE) yr; height, 178.3 ± 2.8 cm; mass, 77.8 ± 2.9 kg; body surface area, 1.95 ± 0.05 m²; peak oxygen uptake ($\dot{V}O_{2peak}$), 50.2 ± 1.6 ml·kg⁻¹·min⁻¹; body fat, 14.0 ± 1.2 %; and skinfold thickness, 2.8 ± 0.5 mm. Subjects had no history of cardiovascular or metabolic disease, or prior cold injuries. All procedures were approved by the appropriate Institutional Review Board.

Preliminary testing. Body density was determined from underwater weighing with percent fat calculated according to Siri (94). Mean skinfold thickness was calculated from 10 sites according to Allen et al. (2). Two weeks before beginning the experimental protocol, all subjects completed an incremental effort cycle ergometer test to exhaustion for determination of $\dot{V}O_{2\text{peak}}$. Briefly, subjects pedaled (60 rpm) at a resistance of 60 watts for 2 min. The resistance was increased 30 watts every 2 min thereafter until the subject reached exhaustion.

Experimental design. Subjects reported to the laboratory one hour before the experiment and instrumentation was affixed. They then sat quietly for 15 minutes on a platform suspended above the water (0700 h T_{amb} , $24.3 \pm 0.2^{\circ}\text{C}$; 1500 h T_{amb} , $24.7 \pm 0.4^{\circ}\text{C}$) while staff obtained pre-immersion measurements of body temperature and metabolic heat production. Following these baseline measures, subjects were quickly lowered into 20°C water to shoulder level where they remained immersed for 120 min. Tests were terminated if T_{re} reached 35°C (45). Each subject completed two immersions on separate days. One experiment began at 0700 h and the other at 1500 h. These trials were separated by at least one week and the order was randomized. Subjects refrained from using alcohol, medications, or tobacco products, and did not exercise for 12 hours before testing. Approximately 1-1.5 h before 0700 h trial, subjects consumed a light breakfast (piece of fruit, juice). Before the 1500 h experiment, subjects ate lunch (sandwich, soda) 3-3.5 h before immersion and were involved in only light activities (i.e., desk work). The rationale for these feedings was to prevent hypoglycemia.

Measurements. Rectal temperature was measured by a thermistor inserted 10 cm past the anal sphincter. Mean weighted skin temperature (\bar{T}_{sk} , °C) and mean weighted heat flow (\bar{H}_c , W·m⁻²) were measured using an integrated disk system (Concept Engineering heat flow sensor with integral linear thermistor, Old Saybrook, CT). Mean weighted skin temperature (°C) was calculated as follows: $\bar{T}_{sk} = 0.06T_{foot} + 0.17T_{calf} + 0.14T_{medial\ thigh} + 0.14T_{lateral\ thigh} + 0.14T_{chest} + 0.07T_{tricep} + 0.07T_{forearm} + 0.14T_{subscapular} + 0.07T_{hand}$ (18). Mean weighted heat flow (W·m⁻²) was calculated as follows: $\bar{H}_c = 0.28H_{subscapular} + 0.14H_{forearm} + 0.08H_{triceps} + 0.22H_{calf} + 0.28H_{thigh}$ (18). Mean body temperature (\bar{T}_b) was calculated as follows: pre-immersion, $\bar{T}_b = 0.8T_{re} + 0.2\bar{T}_{sk}$; during immersion, $\bar{T}_b = 0.67T_{re} + 0.33\bar{T}_{sk}$ (37). Temperature and heat flow measurements were continuously recorded using a computer-automated data acquisition system.

Oxygen uptake ($\dot{V}O_2$) was measured using an automated metabolic analysis system (Model 2900, Sensormedics, Yorba Linda, CA) before and at minutes 5, 25, 45, 65, 85, and 105 of immersion. Metabolic heat production (\dot{M} , W·m⁻²) was estimated from the $\dot{V}O_2$ and respiratory exchange ratio (R) using the following equation (37): $\dot{M} = (0.23[R] + 0.77) \cdot (5.873)(\dot{V}O_2) \cdot (60/A_D)$ where A_D is body surface area (m²), derived from DuBois and DuBois (30).

Blood. Blood samples were drawn before immersion (min 0) and after 90 minutes of immersion via an indwelling venous catheter (18 gauge) placed in a superficial forearm vein. Aliquots were centrifuged at 4°C to separate the plasma. Plasma norepinephrine (NE) was determined (48) in duplicate via high performance

liquid chromatography with electrochemical detection (Model 460, Waters, Inc.).

Plasma samples were frozen at -40°C before analysis.

Statistical Analyses. A 2-way repeated measures analysis of variance (trial X time) was used to determine if significant differences existed between the 0700 h and 1500 h trials. When significant F-ratios were detected, paired comparisons were analyzed post-hoc using the Newman-Keuls test. The slope and intercept of each individual subject's \bar{T}_b - $\Delta\dot{M}$ relationship during immersion was determined by least squares linear regression. Paired t-tests were then utilized to analyze slope and intercept data to determine if differences existed between 0700 h and 1500 h for \bar{T}_b - $\Delta\dot{M}$. Data are reported as means \pm S.E. Significance was accepted at $p \leq 0.05$.

RESULTS

Immersion Time. The immersion time for 7 subjects was the same (120 min) for both trials. However, the immersion times for two of the subjects were lower at 0700 h (56.6 and 66.5 minutes) vs. 1500 h (120 min for both subjects) because they reached the T_{re} safety limit of 35°C .

Temperature and heat flow responses. T_{re} was significantly higher ($p < 0.05$) at 1500 h compared to 0700 h before and throughout the 120 min immersion (Figure 6). The change in T_{re} was greater at 1500 h ($p < 0.05$) from min 60 through min 120 (Figure 6). \bar{T}_{sk} was higher ($p < 0.05$) at min 0 in trial 1500 h ($= 0.4^{\circ}\text{C}$), but after immersion, no differences were observed between trials (Figure 7). The gradient between T_{re} and \bar{T}_{sk} was higher ($p < 0.05$) during trial 1500 h (Figure 7). \bar{H}_c ($\text{W}\cdot\text{m}^{-2}$) during immersion did not differ ($p > 0.05$) between trials (Figure 8).

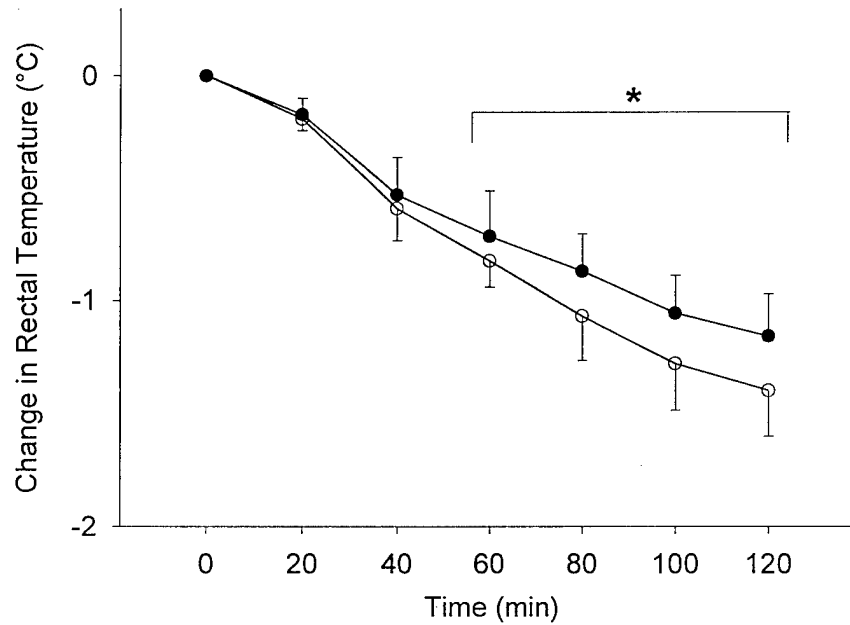
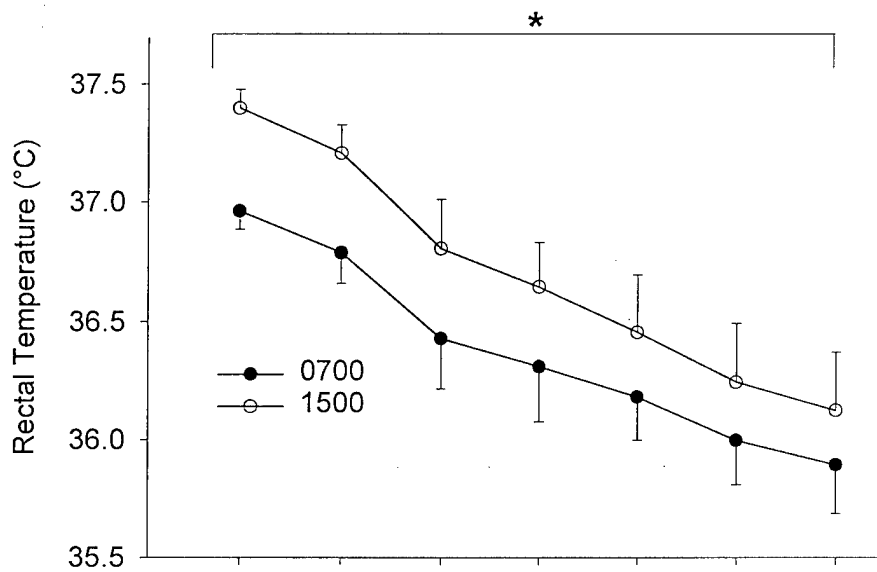


Fig. 6. Rectal temperature and change in rectal temperature vs. time for 0700 and 1500 during cold water immersion. *, Significant difference between 0700 and 1500, $P < 0.05$.

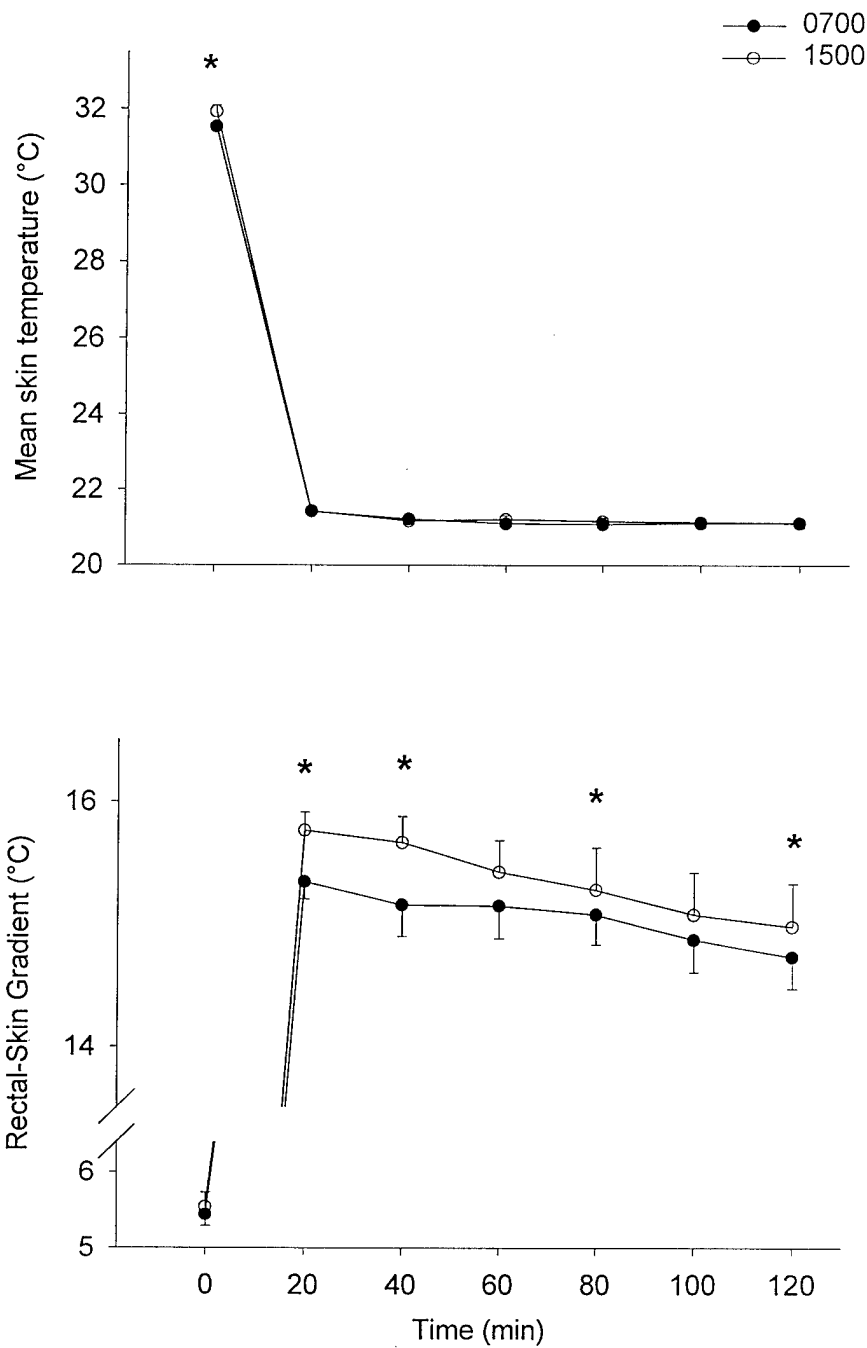


Fig. 7. Mean skin temperature and rectal-skin gradient vs. time for 0700 and 1500 during cold water immersion. *, Significant difference between 0700 and 1500, $P < 0.05$.

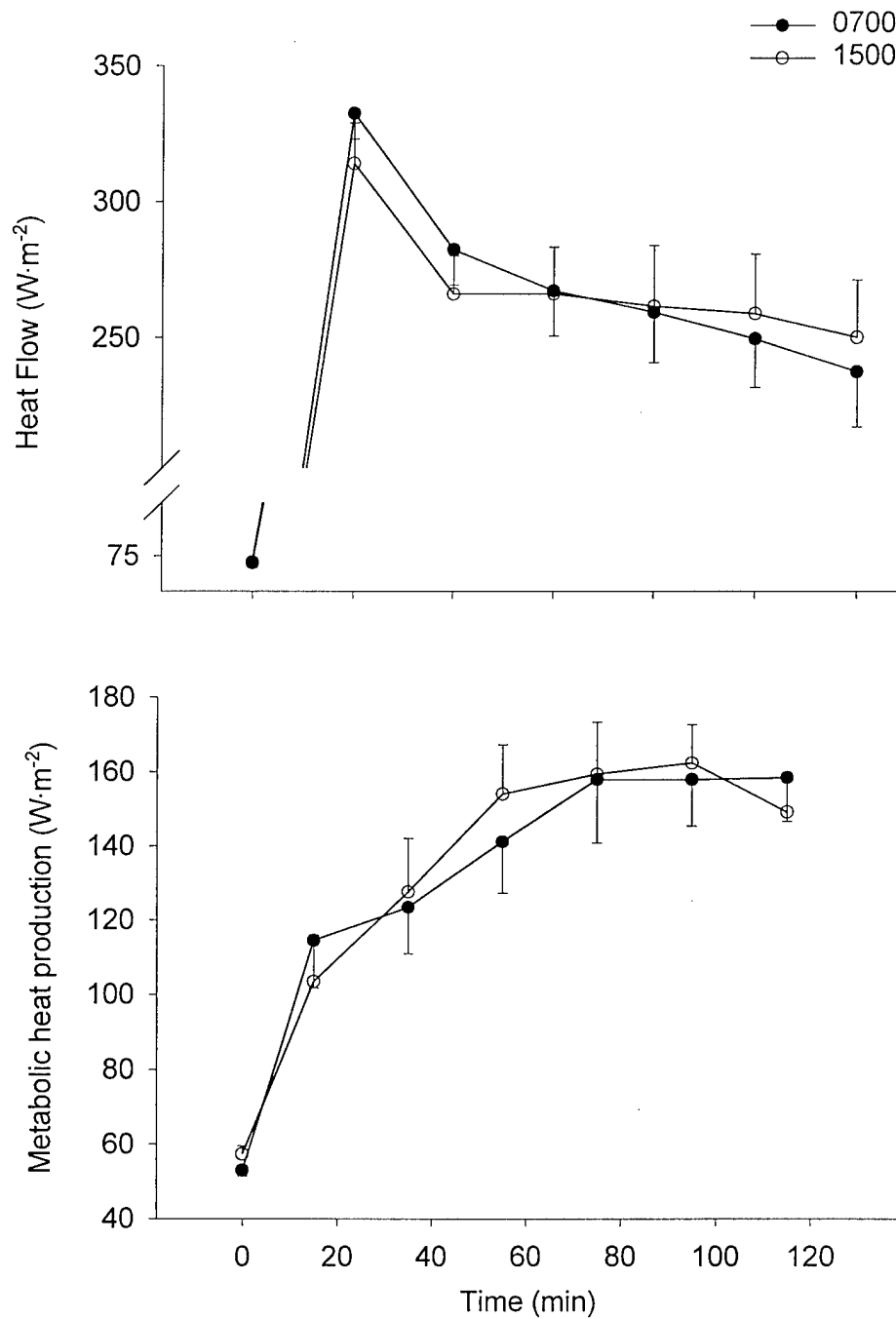


Fig. 8. Heat flow and metabolic heat production vs. time for 0700 and 1500 experiments during cold water immersion.

Metabolic heat production. \dot{M} was similar between trials throughout the 120 min immersion (Figure 8). Analysis of the $\bar{T}_b - \Delta \dot{M}$ relationship demonstrated no differences in either the slope (-64.0 ± 4.2 vs. -68.0 ± 4.9) or intercept (32.7 ± 0.2 vs. 32.9 ± 0.2), for 0700 h and 1500 h respectively.

Plasma norepinephrine. There were no significant differences in plasma NE concentration ($\text{pg} \cdot \text{ml}^{-1}$) at min 0 (290 ± 60 vs. 342 ± 87) and min 90 (1560 ± 448 vs. 1564 ± 361) for 0700 h and 1500 h, respectively.

DISCUSSION

Human heat stress studies have demonstrated a circadian shift in the onset of thermoregulatory responses, similar to the circadian shift in T_{core} (98,100,109). However, this study is the first to focus on whether thermoregulatory responses to cold water immersion are affected by time of day. Overall the data indicate little difference in thermoregulatory responses to acute cold exposure as a function of time of day.

This study found that T_{re} remained higher throughout the immersion period at 1500 h, compared to 0700 h. This is due to the higher initial core temperature at 1500 h vs. 0700 h, a circadian variation well documented (98,100,109). Interestingly, the change in T_{re} during cold water immersion was greater at 1500 h. This is most likely due to the higher $T_{\text{re}} - \bar{T}_{\text{sk}}$ gradient at 1500 h promoting a greater transfer of heat from the core to the periphery. Whether the faster decrease in T_{re} at 1500 h would be sustained after the T_{re} had reached the same temperature as at the end of the immersion begun at 0700 h cannot be determined from this data.

We observed no time of day effect on the two principal physiological responses elicited in humans exposed to cold. Absolute metabolic heat production was similar as were the slope and intercept of the relationship of \bar{T}_b to $\Delta\dot{M}$. This suggests that time of day has no effect on the onset or sensitivity of shivering thermogenesis during cold water immersion. Time of day also appears to have no effect on cold-induced vasoconstriction. Skin temperatures and heat flow during cold water immersion were the same at 0700 h and 1500 h, suggesting no effect on peripheral heat loss. Plasma NE, a marker of sympathetic nervous system activation (32), was also the same between the two times. Together, these observations suggest no effect on the sympathetically mediated vasoconstrictor response to cold. This observation is consistent with those reported from the only other investigation of circadian variations in thermoregulatory effector responses to cooling (103). Tayefeh et al. (103) observed no difference in the cooling-induced vasoconstriction threshold between 0700 h and 1600 h, although a shift was apparent by 0300 h. However it is unclear to what extent that shift resulted from sleep deprivation as opposed to circadian rhythm effects (103). We can only speculate what may occur if we were to perform our experiments at 0300 h.

One potential reason differences in thermoregulatory responses to cold exposure were not observed between 0700 h and 1500 h was that the cold stimulus used was severe. Cold water immersion elicits maximal cutaneous vasoconstriction and high levels of shivering thermogenesis. Thus, it was possible that cold water immersion elicited maximal responses during both trials, and thus "time of day" differences in thermoregulatory responsiveness may have been masked. Therefore, further studies

using a less severe stimulus (cold air) that does not cause maximal constriction and shivering may be warranted.

Two subjects' 0700 h experiments were terminated early because their T_{re} achieved the safety limit of 35°C . For one, there were no differences in metabolic heat production, peripheral heat loss, or cooling rate. It appears that T_{re} reached the safety limit earlier in the 0700 h trial simply due to the 0.6°C lower initial T_{re} compared to 1500 h. Therefore, this subject's overall responses to cold at different times are similar to the other 7 volunteers. The data from the other volunteer whose test was terminated early suggest that a blunted shivering response might have been responsible for the faster drop in core temperature. The slope of this subject's $\bar{T}_b - \dot{M}$ relationship was less and therefore \dot{M} lower at any given \bar{T}_b at 0700 h vs. 1500 h; there was no difference in the intercept (onset) of shivering. The mechanism by which the thermogenic response to cold might have been blunted at 0700 h in this individual is not apparent. Factors known to impair shivering during cold exposure include hypoglycemia (38,74), fatigue (115), and alcohol consumption (36). However, this subject's blood glucose was normal, he was well rested, and had consumed no alcohol before the 0700 cold exposure.

In summary, thermoregulation during cold water immersion is similar between 0700 h and 1500 h. We observed no differences in metabolic heat production, the $\bar{T}_b - \dot{M}$ relationship, or peripheral heat loss. This finding has important implications for the onset of hypothermia ($T_{core} < 35^{\circ}\text{C}$). Individuals typically have a lower resting T_{core} in the morning, thus, when morning cold exposures are severe enough to cause core temperature to decrease, dangerously low core temperature levels may be achieved

sooner than when cold exposure takes place in the afternoon when resting T_{core} is elevated.

PHASE TWO

PRIOR EXERCISE

INTRODUCTION

Exercise has been conjectured to increase an individual's risk of hypothermia during cold exposure (18,25,110). However, experimental and clinical evidence for this is largely anecdotal. Over 30 years ago, Pugh (78,79) concluded that exercise-induced fatigue was an etiologic factor predisposing hikers, climbers, and outdoorsmen to hypothermia, but he provided no data demonstrating this belief with a physiological mechanism for this predisposition. Recently, Thompson and Hayward (105) suggested that exercise during cold-wet exposure may fatigue shivering thermogenesis, but their findings did not definitively support their speculation. Others (68,111) have reported that exercise performed before subsequent cold water immersion exacerbates the fall in core temperature, but these results were inconclusive because pre-immersion core temperature differed between the experiments (68), or a cross-sectional methodology was employed (111). Furthermore, because water has such a high thermal conductivity, peripheral heat loss during cold water immersion may be too pronounced for exercise effects on thermal balance and thermoregulatory effector responses to be detected.

Exercise could increase the risk of hypothermia during subsequent cold exposure due to several reasons. First, exercise might mediate “thermoregulatory fatigue” which would blunt shivering responses and reduce vasoconstriction during subsequent cold exposure. For example, we (115) have observed that a prolonged period of physical exertion coupled with sleep deprivation and negative energy balance resulted in a lowered threshold for shivering despite normal plasma glucose concentrations. Those findings, however, did not allow isolation of the effects of previous exercise from sleep deprivation and negative energy balance. Second, cold exposure immediately after performing leg exercise might result in accentuated heat loss from “thermoregulatory lag”. Thermoregulatory responses are aimed at facilitating heat dissipation during exercise in temperate conditions (85) and subsequent cold exposure might mediate a “lag” in switching from heat loss to conservation. Evidence for this might include increased heat loss from areas of active cutaneous vasodilation such as the torso and arms. Third, exercise might mediate greater heat loss during subsequent cold exposure due to “heat redistribution” to active limbs. During exercise, active skeletal muscle increases perfusion and perfusion can remain elevated for extended durations (104) facilitating regional heat loss over these active limbs during exercise (84). Evidence for a “heat redistribution” might include greater regional heat loss over the active limbs (legs) during subsequent cold exposure.

This study examined whether exercise impairs the body's capability to maintain thermal balance during subsequent cold exposure. It was hypothesized that a greater decrease in core temperature (T_{core}) would occur during cold exposure following exercise compared to cold exposure preceded by resting. We hypothesized that

exercise would mediate some combination of "thermoregulatory fatigue", "thermoregulatory lag", and/or "heat redistribution" which would be manifested as a more rapid cooling rate during cold exposure. To distinguish between these potential mechanisms, and the "thermal" consequences of exercise (increased core temperatures), control experiments were performed following passive heating to elevate the initial core temperature to the same levels achieved by exercise.

METHODS

Subjects. Ten, healthy men volunteered to participate in this study as test subjects. Physical characteristics were age, 24.7 ± 1.7 (SE) yr; height, 176.8 ± 2.1 cm; mass, 78.1 ± 3.5 kg; body surface area, 1.93 ± 0.05 m²; peak oxygen uptake ($\dot{V}O_{2\text{peak}}$), 46.1 ± 1.3 ml·kg⁻¹·min⁻¹; percent body fat, 15.0 ± 1.2 %; and skinfold thickness, 3.2 ± 0.4 mm.

Preliminary testing. Body composition was measured using dual energy x-ray absorbitometry (Model DPX-L, Lunar Corp., Madison, WI). Mean skinfold thickness was calculated from 10 sites according to Allen et al. (2). All subjects completed an incremental cycle ergometer test for determination of $\dot{V}O_{2\text{peak}}$. Briefly, subjects pedaled at 70 watts for 2 min with the resistance increased by 35 watts every 2 minutes until the subject was exhausted and could no longer maintain the exercise intensity.

Experimental Design. Subjects completed two experimental trials, on separate days, spaced by one week. Subjects refrained from smoking, taking medication, and exercising 12 hours before any testing session. Each trial consisted of a standardized cold air test (CAT) preceded by one of two manipulations: A) exercise (EX), or B)

passive heating (HEAT). The EX trial consisted of 60 min semi-recumbent cycle ergometer exercise (EX), immersed to shoulder level in a water immersion pool at $35.0 \pm 0.1^{\circ}\text{C}$ followed by the CAT. The immersion pool holds $\sim 36,000$ liters and is controlled within 0.5°C by a temperature control system. Mean exercise intensity was 55.4 ± 2.3 % $\dot{V}\text{O}_{2\text{ peak}}$ for EX. The HEAT trial consisted of sitting in the immersion pool at $38.2 \pm 0.0^{\circ}\text{C}$ until rectal temperature rose to match that at the completion of EX followed by the CAT. This approach precluded using a randomized design and the HEAT trial always followed the EX trial. Immediately following EX or HEAT, subjects towed off, changed into dry shorts and socks, and were taken to the anteroom of the cold chamber for baseline measurements. This took approximately 20 minutes. Five minutes of baseline data (body temperatures, HR, metabolic rate) were collected outside the cold air chamber ($22.8 \pm 0.8^{\circ}\text{C}$) while the subject sat quietly, and then they rose and walked into the cold air chamber ($4.6 \pm 0.1^{\circ}\text{C}$) and reclined for up to 120 min in a nylon mesh lounge chair. While reclining, the subjects sat quietly and were not allowed to employ behavioral thermoregulation. The trials were all conducted at the same time of day to control for the potential influence of circadian rhythmicity.

Measurements and Calculations. Rectal temperature (T_{re}) was measured by a thermistor inserted 10 cm past the anal sphincter. Integrated heat flow and skin temperature disks (Concept Engineering, Old Saybrook, CT) were secured at 5 (in water) and 8 (CAT) sites (right side of the body). Mean weighted skin temperature (\bar{T}_{sk}) during water immersion was calculated as follows: $\bar{T}_{sk} = 0.28T_{\text{subscapular}} + 0.14T_{\text{forearm}} + 0.08T_{\text{triceps}} + 0.22T_{\text{calf}} + 0.28T_{\text{lateral thigh}}$. During CAT, \bar{T}_{sk} ($^{\circ}\text{C}$) was calculated as follows: $0.06T_{\text{foot}} + 0.17T_{\text{calf}} + 0.28T_{\text{lateral thigh}} + 0.14T_{\text{chest}} + 0.07T_{\text{tricep}} + 0.07T_{\text{forearm}} +$

$0.14T_{\text{subscapular}} + 0.07T_{\text{hand}}$. Mean weighted heat flow ($\bar{H}\bar{F}$, $\text{W}\cdot\text{m}^{-2}$) was calculated as follows: $0.06H\bar{F}_{\text{foot}} + 0.17H\bar{F}_{\text{calf}} + 0.28H\bar{F}_{\text{lateral thigh}} + 0.14H\bar{F}_{\text{chest}} + 0.07H\bar{F}_{\text{triceps}} + 0.07H\bar{F}_{\text{forearm}} + 0.14H\bar{F}_{\text{subscapular}} + 0.07H\bar{F}_{\text{hand}}$. Tissue insulation was calculated as follows: $I_T = (T_{\text{re}} - \bar{T}_{\text{sk}}) / \bar{H}\bar{F}$ (42). Mean body temperature (\bar{T}_b) was calculated as follows: pre-CAT, $\bar{T}_b = 0.8T_{\text{re}} + 0.2\bar{T}_{\text{sk}}$, during CAT, $\bar{T}_b = 0.67T_{\text{re}} + 0.33\bar{T}_{\text{sk}}$ (108). Temperature and heat flow measurements were made continuously using an automated data acquisition system.

Oxygen uptake ($\dot{V}O_2$) was measured using an automated metabolic measurement and analysis system (Model 2900, Sensormedics, Yorba Linda, CA) at minutes 0 (baseline) and 30 during the water immersion. During CAT, $\dot{V}O_2$ was measured at minutes 0 (baseline), 15, 35, 55, 75, 95, and 115. Metabolic heat production (M , $\text{W}\cdot\text{m}^{-2}$) was estimated from the $\dot{V}O_2$ and respiratory exchange ratio (RER) using the following equation (37): $M = (0.23[\text{RER}] + 0.77) \cdot (5.873)(O_2) \cdot (60/A_D)$ where A_D (30) is body surface area (m^2).

Cumulative body heat debt was defined as the total negative heat storage integrated over time and expressed as a positive number. Body heat storage (\dot{S} , $\text{W}\cdot\text{m}^{-2}$) was calculated: $\pm \dot{S} = -\dot{W} - \dot{L} - \dot{K} - \dot{E} - (\dot{R} + \dot{C})$ where \dot{M} is the metabolic rate, \dot{W} is work rate (0 in this experiment), \dot{L} is the respiratory heat losses by convection and evaporation, \dot{E} is evaporative heat loss (set at $4.1 \text{ W}\cdot\text{m}^{-2}$ in this experiment), \dot{K} represents conductive heat loss (0 in this experiment) and $\dot{R} + \dot{C}$ represents dry heat loss, measured by heat flow disks (37, 108).

Blood was drawn from an indwelling venous catheter (antecubital) in the left arm before beginning the CAT (min 0) and at minutes 15, 30, 60, 90, and 120 during CAT.

Catheter patency was maintained between blood draws by injecting heparinized saline into the catheter. Blood samples were analyzed to determine plasma glucose concentration using an auto analyzer (Model 2300, Yellow Springs Instrument, Inc.) to ensure that subjects maintained euglycemia. Plasma norepinephrine (NE) was determined by gas chromatography (118).

Statistical Analyses. Data were analyzed using a 2-way repeated measures analysis of variance. When significant F-ratios were calculated, paired comparisons were made post-hoc using Newman-Keuls tests. The slope and threshold of each individuals \bar{T}_b vs. $\dot{\Delta M}$ relationship was determined by least squares linear regression. Paired t-tests were used to determine if differences in slope or intercept data existed between EX and R for \bar{T}_b vs. $\dot{\Delta M}$. Data are reported as means \pm S.E. Significance was accepted at $p < 0.05$.

RESULTS

Water Immersion. All subjects completed 60 min of cycling during EX. The mean immersion time required during HEAT to match the T_{re} rise observed during EX was 53.4 ± 5.0 min. The mean T_{re} at the end of the immersion periods were $38.19 \pm 0.14^\circ\text{C}$ and $38.08 \pm 0.10^\circ\text{C}$, during EX and HEAT, respectively ($P > 0.05$). The average $\dot{V}O_2$ ($\text{L}\cdot\text{min}^{-1}$) during immersions were 1.97 ± 0.12 and 0.34 ± 0.02 , for EX and HEAT, respectively ($P < 0.05$). For EX, this $\dot{V}O_2$ corresponded to $55.4 \pm 2.3\%$ of the measured $\dot{V}O_{2\text{ peak}}$. Final heart rates ($\text{beats}\cdot\text{min}^{-1}$) during immersion were 149.3 ± 6.1 and 102.1 ± 3.1 , for EX and HEAT, respectively ($P < 0.05$). Weight loss (kg) from sweat was 1.07 ± 0.15 and 1.06 ± 0.18 during EX and HEAT, respectively ($P > 0.05$).

Rectal temperature (CAT). During the transition from the immersion pool to the cold air chamber, T_{re} fell during HEAT. Therefore, T_{re} at min 0 was slightly, but significantly higher (0.14°C , $P < 0.05$) in EX vs. HEAT (Figure 9). By the 10th min of cold air exposure, differences between trials were no longer apparent. However, by the 40th min of CAT, T_{re} had fallen lower ($P < 0.05$) during EX compared to HEAT and the difference between trials grew larger as exposure continued to the 120th min. The cooling rate ($^{\circ}\text{C}\cdot\text{h}^{-1}$) from min 10 to the end of the exposure was faster ($P < 0.05$) for EX (-0.64 ± 0.07) than HEAT (-0.57 ± 0.04).

Skin temperature (CAT). The \bar{T}_{sk} and the $T_{re}-\bar{T}_{sk}$ gradient are shown in Figure 10. Cold air exposure caused \bar{T}_{sk} to decrease until a new steady state value of $\sim 23^{\circ}\text{C}$ was achieved. There was a concomitant increase in the $T_{re}-\bar{T}_{sk}$ gradient during CAT. The apparent tendency for higher \bar{T}_{sk} and lower $T_{re}-\bar{T}_{sk}$ in EX, vs. HEAT, during the last 60 min of the cold exposure did not achieve statistical significance.

Heat flow (CAT). \bar{HF} was higher ($P < 0.05$) during CAT in EX vs. HEAT (Figure 11). Also I_T during CAT was lower ($P < 0.05$) in EX compared to HEAT (Figure 11). Individual site HF and I_T are presented in Figure 12. Calf HF and I_T demonstrated a significantly ($P < 0.05$) greater HF and lower I_T between EX and HEAT. Hand HF also tended ($p = 0.06$) to be higher in EX.

Metabolic heat production & heat debt (CAT). Metabolic heat production did not differ between EX and HEAT at any time throughout CAT. The final \dot{M} at min 115 was 146.6 ± 6.5 and $136.1 \pm 3.6 \text{ W}\cdot\text{m}^{-2}$ for EX and HEAT, respectively. The relationships (slope and intercept) between mean body temperature (\bar{T}_b) and the corresponding increment in metabolic heat production over pre-CAT values ($\Delta\dot{M}$, a measure of

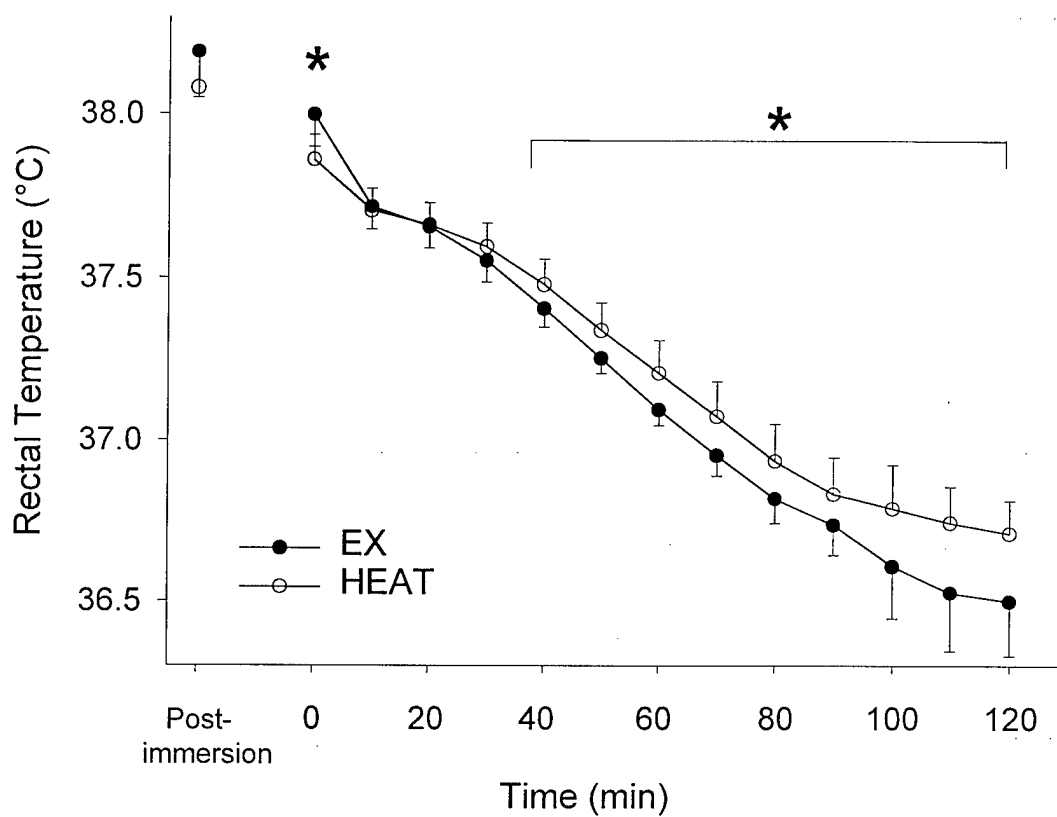


Fig. 9. Rectal temperature vs. time for exercise and passive heating experiments during cold air exposure. Postimmersion, temperature at end of water immersion. *, Exercise significantly different ($P < 0.05$) than control at specified times.

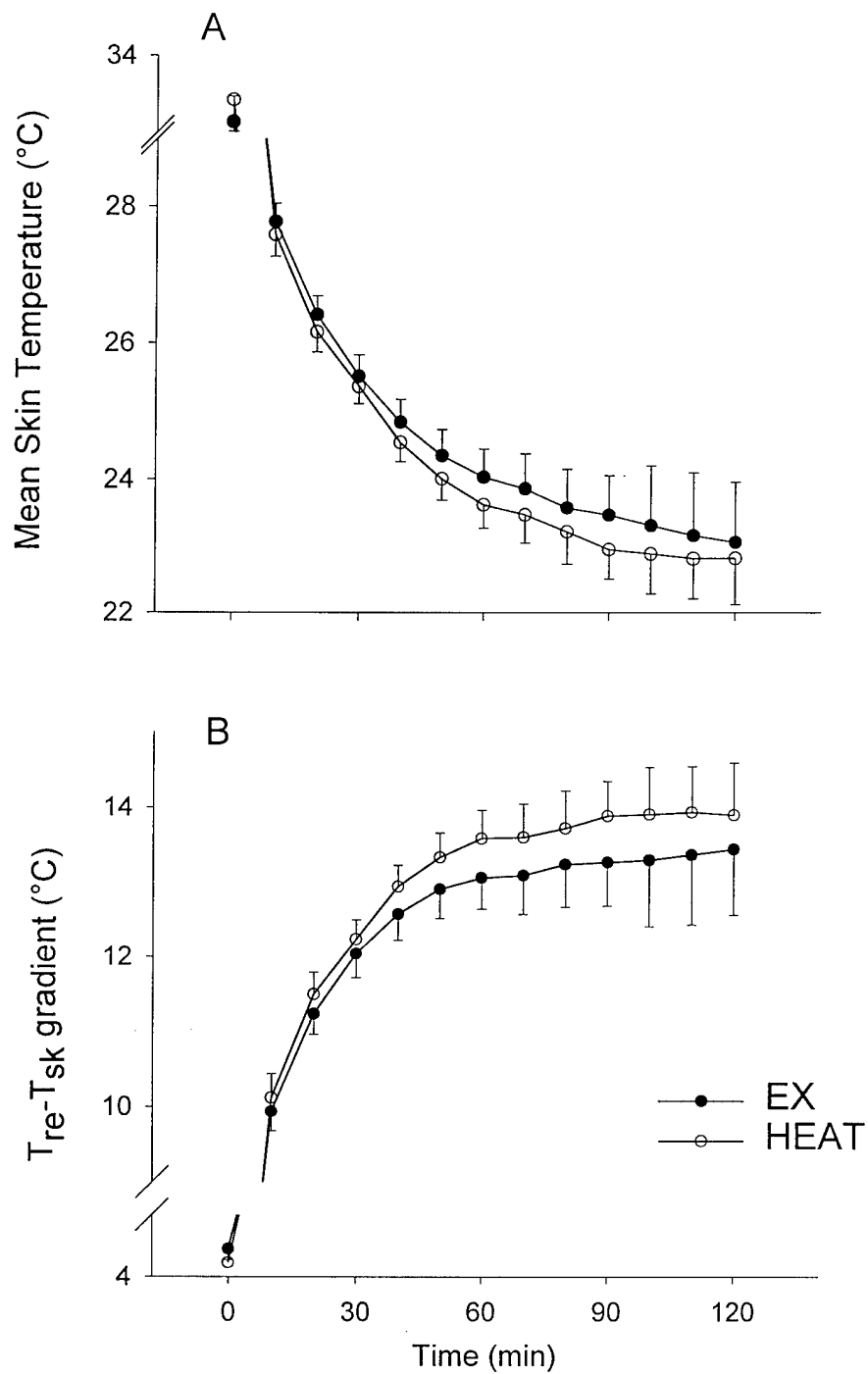


Fig. 10. Mean skin temperature (A) and rectal-skin temperature gradient (B) vs. time for exercise and passive heating experiments during cold air exposure.

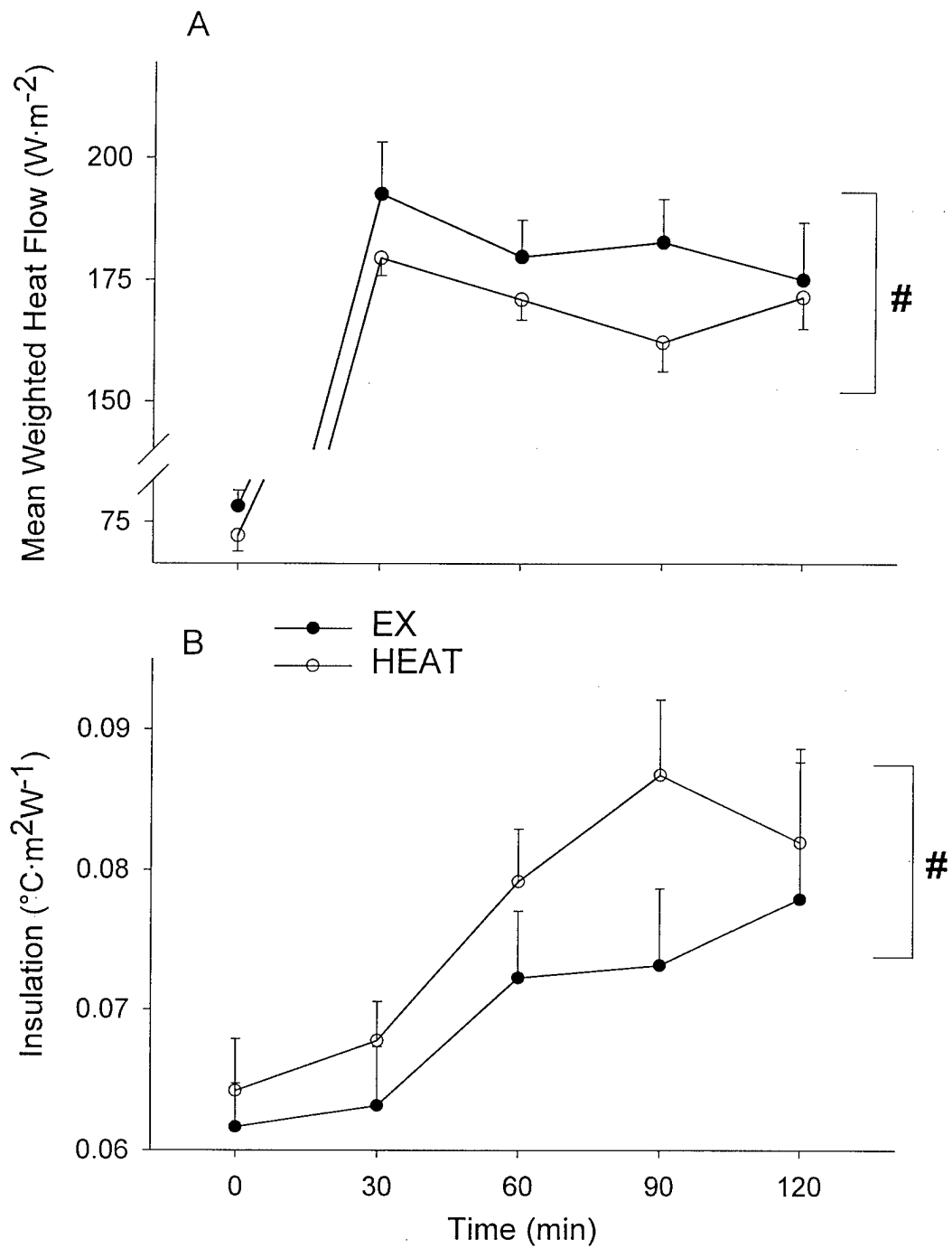


Fig. 11. Mean weighted heat flow (A) and insulation (B) vs. time for exercise and passive heating experiments during cold air exposure. #, Main effect, EX significantly different from Control, $P < 0.05$.

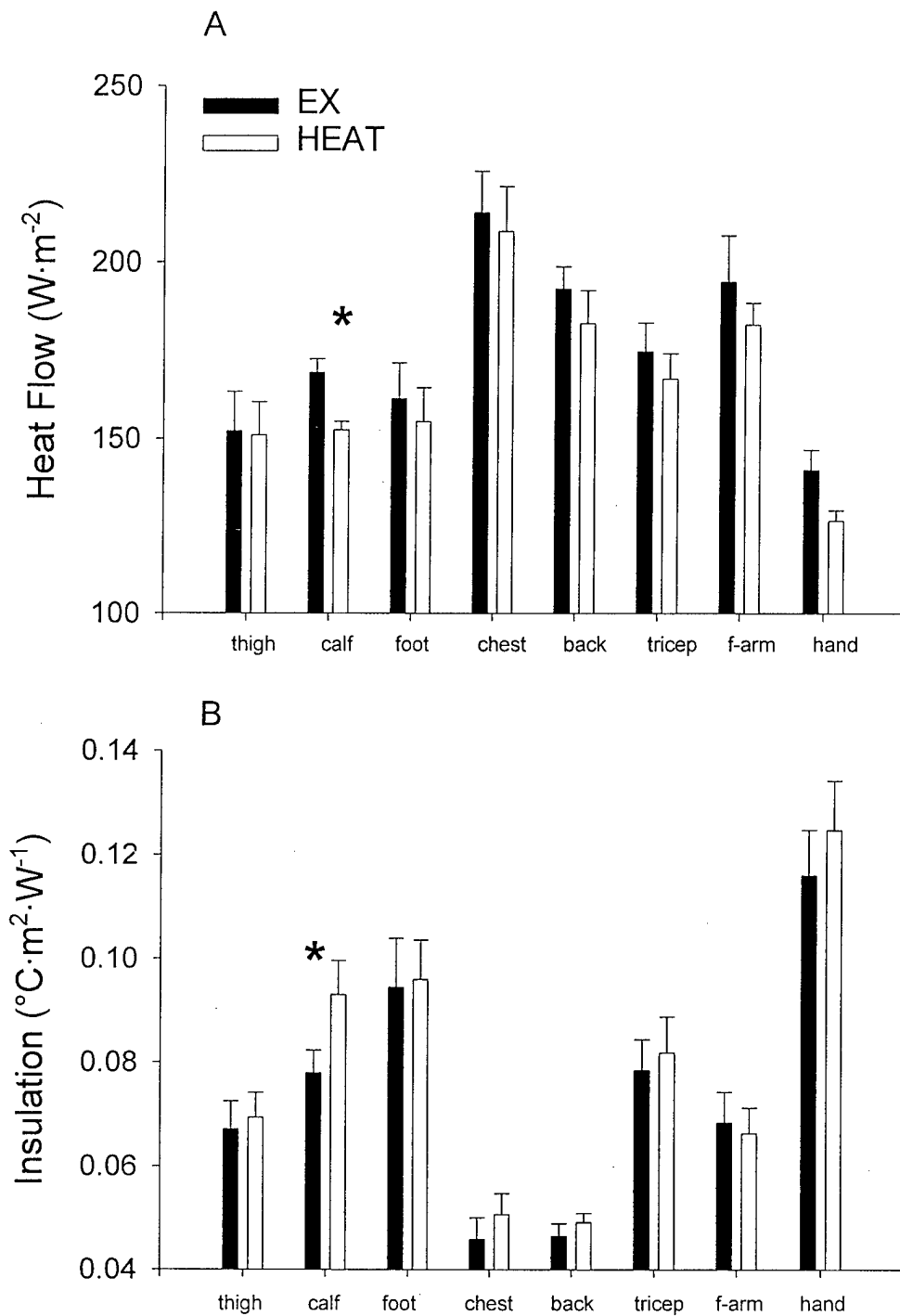


Fig. 12. Individual heat flow (A) and insulation (B) for 8 sites measured. *, calf heat flow was higher ($P < 0.05$) and insulation lower ($P < 0.05$) during EX.

shivering thermogenesis) did not differ between trials. Slopes ($\text{W}\cdot\text{m}^{-2}\cdot^{\circ}\text{C}^{-1}$) were -33.8 ± 3.0 and -32.7 ± 3.4 for EX and HEAT, respectively. Intercepts ($^{\circ}\text{C}$) were 34.5 ± 0.2 and 34.3 ± 0.1 for EX and HEAT, respectively. Cumulative heat debt ($\text{W}\cdot\text{m}^{-2}$) was not different between EX (547.5 ± 47.0) and HEAT (532.9 ± 28.5) after 120 min of exposure.

Plasma glucose, norepinephrine (CAT). Plasma glucose concentrations were not affected by CAT in either trial and there were no differences between trials. Glucose values averaged between $4\text{--}6 \text{ mmol}\cdot\text{L}^{-1}$ throughout CAT. Plasma norepinephrine concentrations increased from $2.5 \text{ nmol}\cdot\text{L}^{-1}$ to $10\text{--}15 \text{ nmol}\cdot\text{L}^{-1}$ during cold air exposure, with no differences between EX and HEAT.

Heart rate (CAT). HR tended ($P = 0.06$) to be higher from min 30-75 ($\sim 10 \text{ beats}\cdot\text{min}^{-1}$) in EX during CAT compared to HEAT.

DISCUSSION

This study determined if exercise pre-disposes people to experience a greater decline in core temperature during subsequent cold exposure. An expected response to exercise, even in temperate climates, is an increased core temperature (85). Therefore, to isolate effects of body heat content and temperature changes from other exercise effects (thermoregulatory fatigue, thermoregulatory lag, heat redistribution), control experiments were needed in which initial pre-cold exposure core temperature values had been passively elevated to the same value as measured post-exercise. If such controls were not employed, the $T_{\text{core}}\text{--}T_{\text{skin}}$ gradient would be greater during cold exposure after exercise and subsequently heat loss would be facilitated. In addition, the absolute core temperatures could not be compared between trials beginning with

different initial values. However, it is experimentally difficult to match both core and skin temperature increases during exercise in air to increases induced by passive heating in air, especially if the durations of the interventions are also desired to be similar.

Matching of core and skin temperature changes during rest (passive heating) and exercise sessions of similar duration are better accomplished by using water immersion.

The exercise intensity ($55\% \dot{V}O_{2peak}$) was selected to represent moderately strenuous, fatiguing activities.

The primary finding from this study was that when individuals exercised before cold exposure, they cooled faster than when rest preceded cold exposure. However, the data are not consistent with our hypothesis that exercise would lead to “thermoregulatory fatigue” of the shivering response to cold. We had based that hypothesis on our own findings (17) and those reported by others (78,79,105) suggesting that shivering can become fatigued. In this study, the shivering response to cold was the same whether or not exercise preceded the cold exposure. In contrast, mean weighted heat flow measurements were higher and, concomitantly, tissue insulation less during cold exposure following exercise. Skin temperatures during cold air exposure also tended to be higher ($0.2\text{-}0.5^{\circ}\text{C}$) following exercise. Collectively, these observations indicate that, following exercise, greater peripheral heat loss from the skin (“thermoregulatory lag” and/or “heat redistribution”) was responsible for the greater cooling rates during cold exposure.

Several factors might explain why peripheral heat loss during cold exposure was greater when preceded by exercise than passive heating. One possibility is that post-exercise hyperemia in the leg muscles persists during cold exposure, increasing

convective heat transfer from the body's core to the periphery overlying active muscle relative to cold exposure preceded by rest ("heat redistribution"). The higher heat flow and lower insulation in the calf during cold exposure following exercise, compared to passive heating, are consistent with this explanation. Another possibility is that the prior exercise blunted the drive for vasoconstriction normally elicited in response to cold ("thermoregulatory lag"). However, cold-induced vasoconstriction is sympathetically mediated, and the norepinephrine response to cold, considered reflective of sympathetic nervous activation (32), was the same whether cold exposure was preceded by exercise or passive heating. On the other hand, sensitivity of peripheral arterioles to NE released in response to cold might be diminished following exercise (51).

Our results contrast those reported by Kenny et al. (58) who found that the threshold for vasoconstriction was elevated after exercise. They suggested that exercise would result in the retention of heat during subsequent recovery in a cold environment (58). However, our subjects exercised for one hour in water while those studied by Kenny et al. (58) only completed a short exercise bout (15 min) and thus our subjects may have been more fatigued. In addition, Kenny et al. (58) did not control for differences in initial core temperature before cold exposure, but we matched initial T_{core} values before cold exposure between our trials. Finally, our volunteers were subjected to a whole body cold air exposure at a constant temperature compared to the water-perfused suit that Kenny et al. (58) used. Thus methodological differences probably account for discrepant observations in our study and that of Kenny et al. (58).

Although we observed a lower T_{core} when cold exposure followed exercise as well as significantly higher peripheral heat flows and a tendency for higher metabolic heat production, compared to cold exposure following passive heating, we found no statistical difference in cumulative heat debt, measured by partitioned calorimetry. There was a tendency for an increased metabolic heat production during cold exposure following exercise as compared to exposures following passive heating which probably offset the increased heat flow. Therefore, the greater fall in T_{core} during cold exposure following exercise may reflect a redistribution of body heat content (65,66) from the core to the periphery due to a higher peripheral blood flow during and for some time after exercise (49).

The absence of an exercise effect on shivering thermogenesis suggests that this response to cold is not easily fatigable. We observed no difference in the \bar{T}_b vs. $\Delta \dot{M}$ relationship between trials suggesting that the differences in T_{re} between trials were not due to a change in central control of shivering thermogenesis. Perhaps exercise intensity and duration were not sufficient to fatigue the shivering mechanism, which is a relatively low intensity activity (116), at least compared to exercise. In Pugh's case report of the Four Inn's Walk (79), the participants were exercising up to 20-h in cold-wet conditions. Likewise, the subject in Thompson and Hayward's study (105) who developed shivering fatigue was exercising for 4-h in severe cold-wet conditions. Another possibility is that shivering impairments observed in these earlier studies may not reflect fatigue, but rather hypoglycemia, which is known to impair shivering (38,74). Plasma glucose levels were not measured in those previous studies (79,105). In our study, plasma glucose concentrations remained normal throughout cold exposure.

A possible limitation to extrapolating our results to non-immersed exercise relates to the potential effects of immersion, especially immersion associated alterations in hormonal responses to exercise compared to exercise in air. However, during cycle exercise at $\sim 60\% \dot{V}O_{2\text{peak}}$, performed in water to the neck, there were no difference in catecholamine responses compared to cycling in air at the same intensity (22). Another study (89) also demonstrated that plasma osmolality, which is known to affect central temperature regulation (93), was not different during exercise at $60\% \dot{V}O_{2\text{peak}}$ in water and air. In fact, hormonal responses to exercise have been found not to differ between air and immersion (89) with the exception that plasma renin activity was lower and plasma atrial natriuretic peptide (ANP) higher during water exercise vs. air exercise at $60\% \dot{V}O_{2\text{peak}}$. However, there is no known influence of renin and ANP on hypothalamic neurons regulating thermoregulatory responses to cold. Studies comparing thermoregulatory responses to cold subsequent to exercise in air are warranted to confirm our findings. However, it seems reasonable to conclude that our data indicate that, after exercise, the ability to maintain thermal balance in the cold may be compromised.

This study was the first to examine the possibility that acute exercise performed before whole body cold air exposure impairs the ability to maintain thermal balance, as others have speculated. Our findings demonstrated that exercise before cold air exposure may lead to a greater fall in core temperature due to reduced insulation and increased heat loss and a redistribution of heat from the core to the periphery. The data also suggest that an exercise-related factor ("heat redistribution") led to the greater fall in core temperature and not the rise in core temperature that accompanies exercise.

These findings may also have potential implications for people who exercise hard and then are exposed to cold stress, or people who exercise hard outdoors in the cold and then stop, but do not return indoors immediately.

IMMUNE RESPONSES

INTRODUCTION

Both anecdotal (19,50) and experimental reports (6,62) have suggested that cold exposure may increase an organism's susceptibility to infection (91). Cold-induced decrements in immunosurveillance can be a particular problem for winter athletes or for military personnel who must pursue physical activities in cold environments. Upper respiratory tract infections appear to be the main cause of illness and reasons for missed practice in elite cross-country skiers (7). Studies of sustained military operations in the Canadian Arctic, also, have reported an increased incidence and severity of upper respiratory tract infections during patrols involving high levels of energy expenditure and exposure to cold conditions both day and night (83,96). As the effective accomplishment of both athletic endeavors and military operations depends on a high level of human performance, maintenance of health is essential in situations where athletes or troops are exposed to adverse environmental conditions.

It remains unclear whether human viral susceptibility during and following cold exposure is attributable to a cold-induced change in the function of the immune cells or to other incidental consequences of the cold environment such as drying of the mucosal surface, a slowing of tracheal ciliae (41) or a deterioration of the normal barrier function

of the skin (44). Animal studies have demonstrated that cold exposure induces changes in both cellular and humoral aspects of immune function (91), including a reduction in NK cell count and cytolytic activity (1,112), a decrease in lymphocyte proliferation (43,92) and (after several days of cold exposure) an enhanced production of pro-inflammatory cytokines (54). However, in many of these studies, the animals were subjected to prolonged periods of cold exposure or were placed in environments to which they were unaccustomed (for example, cold water immersion). This would have placed additional stress on the animals studied and the psychological stress rather than the cold exposure may have influenced their immune status. Moreover, the results obtained in animal studies are difficult to apply to humans due to inter-species differences.

To date, there has been minimal research examining how cold exposure affects immune function in humans and no one has examined how any changes may be modulated by prior passive heating or moderate exercise (with or without a thermal clamp - a procedure which maintains a constant core body temperature). It is known that passive heating and exercise each recruit leukocytes (granulocytes, lymphocytes and lymphocyte subsets) into the circulation and enhance NK cell function (10). Immersion of healthy young men in cold (14°C) water induces a leukocytosis (53). Furthermore, a brief bout (30 min) of cold (4°C) air exposure increases NK cell activity (60). We thus hypothesized that exposure to cold air (5°C) for 2 h would have immunostimulating effects and that pre-treatment with exercise or passive heating would have algebraically additive effects on this response.

METHODS

Subjects. The subjects were seven healthy, moderately fit males aged 20 - 34 yr [mean (SE) = 24.0 (1.9) yr] recruited from a pool of military personnel in accord with a

protocol approved by the Human Experimentation Committee at the US Army Research Institute of Environmental Medicine. Written informed consent was obtained prior to participation in the study. Exclusionary criteria included acute infection, and a history of allergic conditions, prior cold injury, Raynaud's syndrome, cardiovascular disease, respiratory disease or diabetes mellitus. Subjects were asked to refrain from consumption of alcohol, smoking, taking medication, and exercising 12 hours prior to any testing session. Subjects were of average male height [1.76 (0.02) m], body mass [79.4 (4.7) kg], percent body fat [14.6 (1.6) %] and aerobic power [45.7 (2.0) mL·kg⁻¹·min⁻¹].

Experimental Design. Each subject visited the laboratory on 5 occasions. At entry, anthropometric measurements (height, body mass and determination of percent body fat using dual energy x-ray absorbitometry ["DEXA"- Model DPX-L, Lunar Corp., Madison, WI]) were made and peak oxygen consumption ($\dot{V}O_{2peak}$) was determined using a progressive cycle ergometer test. The cycle ergometer protocol consisted of a warm-up (70 W for 2 min) followed by a 35 W increase in exercise intensity every 2 min until volitional exhaustion.

Subjects then completed four separate trials with different pre-treatments followed by a standardized cold air exposure. The pre-treatments took place in a water bath, whereby subjects (submerged to the shoulder) either remained seated or performed semi-recumbent exercise, pedaling (38-45 rpm) on a modified Monark cycle ergometer (88). A weighted waist belt allowed subjects to maintain their position underwater.

In the control condition, each subject sat at rest in approximately thermoneutral conditions (a 35°C water bath) for 60 min. During passive heating, each subject sat in a warm (38°C) water bath until their rise in core temperature matched that which had occurred during exercise in 35°C water (times ranging from 38 to 70.5 min, with an average of 59.8 min). During one pre-treatment involving exercise, subjects performed cycle ergometer exercise for 60 min at an average of 55 % of their $\dot{V}O_{2peak}$ while

immersed in cold water (18°C); this maintained core body temperature constant (the “thermal clamp” condition). For the pre-treatment involving exercise without a thermal clamp, each subject performed cycle ergometer exercise for 60 min at an average of 55 % of their personal $\text{VO}_{2\text{peak}}$ while immersed in water at 35°C. Immediately following each pre-treatment, the subjects were dried and dressed in dry cotton shorts and cotton socks (0.3 clo). Within 20 min, subjects were then transferred to a cold climatic chamber and exposed to cold air (5°C, 40 % relative humidity, windspeed of 0.7 m·sec⁻¹) for 120 min. Subjects were assigned to each pre-treatment according to the following order (exercise in 35°C water; sitting in warm water; exercise in cold water; sitting in thermoneutral water). This was done in order to match the rise in core temperature between the exercise trial (exercise in 35°C water) and the resting trial (sitting in warm water). Each experiment took place at the same time of day; individual experiments being separated by one-week intervals.

Physiological Measurements. Heart rate (ECG, CM-5 configuration) and rectal temperature (rectal thermistor inserted 0.1 m past the anal sphincter) were measured continuously during both water immersion and cold exposure. To avoid subject discomfort and resultant mouthpiece leakage, oxygen uptake was measured intermittently (after 15-20 min of immersion and at regular intervals during cold exposure), using an on-line metabolic analysis system (SensorMedics, Model 2900, Yorba Linda, CA).

Blood Sampling. Venous blood samples were collected from an indwelling venous catheter (Deseret Medical, Sandy, USA) that had been inserted into the medial antecubital vein of the left arm 30 min prior to each pre-treatment. Patency of the catheter was maintained between sampling by means of a 1.0 mL heparin-saline lock (100 units·mL⁻¹). Blood samples of 18 mL volume were taken prior to (pre) and at the end of exercise (post) and 23 mL samples were taken prior to (0 min), during (60 min) and at the end of cold exposure (120 min). The first 1.0 mL of each blood sample was

discarded. Hemoglobin and complete blood counts were checked at the beginning of each experiment. All values proved normal, but the study design provided for more detailed hematological evaluation and a delaying of further experiments if a hematological deficit had persisted as a result of blood sampling during a prior treatment.

At the specified times, aliquots of blood were drawn into vacutainers (Becton-Dickinson, Oakville, Ont., Canada). Complete blood counts (CBC) were determined on tripotassium ethylenediamine tetra-acetate (K₃EDTA)-treated specimens of whole blood using a Cell Dyn Hematology System (Cell Dyn 3500, Abbott Laboratories, Abbott Park, IL, USA). Heparinized whole blood (143 USP units sodium heparin) was used for the cytolytic functional assay. A separate vacutainer containing K₃EDTA-treated blood provided plasma samples for cytokine and cortisol analyses. Aliquots of blood for catecholamine analyses were drawn into 4.5-mL vacutainers containing K₃EDTA and reduced glutathione (Amersham, Arlington Heights, IL, USA). Catecholamine and cortisol samples were obtained only during the cold exposure component of the experiment. The method of Dill and Costill (27) was used to make appropriate adjustments of white cell counts, cytokine and hormonal concentrations for changes in blood and plasma volumes relative to their respective baseline values.

Immunophenotyping. Immunophenotyping was performed using 100 μ L samples of K₃EDTA-treated whole blood. Samples were first washed with 2 mL of phosphate buffered saline containing 0.1% sodium azide (0.1% NaN₃-PBS). The cell pellet was then stained with 10 μ L of the selected monoclonal antibodies (mAb, Becton-Dickinson) conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin chlorophyll protein (per CP) in the following staining combinations: anti-CD3 mAb (FITC)/ anti-CD19 mAb (PE), anti-CD4 mAb (FITC)/ anti-CD8 mAb (PE), anti-CD4 mAb (FITC)/ anti-CD8 mAb (PE)/ anti-CD 3 (per CP) and anti-CD3 mAb (FITC)/ anti-CD16,56 mAb (PE) (Becton Dickinson, Mississauga, Ont., Canada). After 30 min of incubation

on ice in the dark, 2 mL of 10% FACS lysing solution (Becton Dickinson, Mississauga, Ont., Canada) was added and the tubes were vortexed. The tubes were then kept in the dark at room temperature (23°C) for a further 10 min to lyse the red cells. Non-lysed cells were separated by centrifuging at 300 *g* for 5 min at 4 °C. Tubes were then washed twice with 2 mL of a cold 0.1 % NaN₃-PBS solution and were centrifuged for a further 5 min at 4°C and 300 *g* after each wash. The resultant pellet was re-suspended in 0.3 mL of cold 0.1 % NaN₃-PBS with 3 % formaldehyde, vortexed and later analyzed by flow cytometer.

A FACS flow cytometer (Becton-Dickinson Immunocytometry Systems, Mountainview, CA, USA) was used to examine the mAb-stained cell suspensions. Firstly, the flow cytometer was calibrated with a mixture of mono-sized FITC- and PE-conjugated and unconjugated latex particles (~6.0 µm Calibrite beads, Becton Dickinson) using FACScomp software (Becton-Dickinson Immunocytometry Systems). An isotype negative control was used to optimized the setting of the fluorescence detectors for each subject. Fluorescence compensation was adjusted using a dual stained anti-CD4 mAb (FITC)/anti-CD8 mAb (PE) sample. Gate settings for the lymphocyte population and boundaries for fluorescence intensity were determined by non-specific staining, using control tubes containing whole blood and mouse immunoglobulins IgG_{2a} mAb (FITC) and IgG₁ mAb (PE). The absolute count for a given lymphocyte subset was estimated by multiplying the observed percentage for that subset by the total number of lymphocytes in the peripheral blood, the latter value being adjusted for changes in blood volume.

Determination of NK Cell Activity. A ⁵¹Cr release assay assessed the total cytolytic activity of isolated PBMC. Peripheral blood mononuclear cells (PBMC) were first isolated by Ficoll-Hypaque centrifugation. 10 mL of heparinized venous blood was diluted with an equal volume of Dulbecco's phosphate-buffered saline (PBS) (Sigma

Chemical Co., St. Louis, MO, USA). For each blood sample, three 15 mL centrifuge tubes were used to layer 7 mL of diluted blood carefully over 5 mL of Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). The suspension was then centrifuged for 30 min at 20°C and 450 *g*. The mononuclear layer was removed and washed twice, firstly with PBS and then with RPMI-1640 culture medium containing L-glutamine (Gibco, Burlington, Ont., Canada). The cell suspension was centrifuged for 10 min at 10°C and 275 *g* after each wash. The washed peripheral blood mononuclear cells were re-suspended in 1 mL of RPMI-1640, supplemented with 10 % fetal calf serum (Gibco) (10 % FCS RPMI-1640). The total PBMC count was determined by means of an electronic cell counter (Coulter Counter Model ZM, Luton, Beds, England) and was then adjusted to 2×10^6 cells·mL⁻¹, using 10% FCS as the diluent.

A human erythroleukemic cell line (K562: American Type Culture Collection, Rockville, Md. USA), maintained in suspension in our laboratory, was used for the ⁵¹Cr-release assay. One million K562 cells, maintained in 10 % FCS RPMI-1640 medium (Gibco) were labeled by mixing with 100 µL (3.7 MBq) of sodium chromate-51 (⁵¹Cr) (New England Nuclear, Boston, MA, USA) for 60 min at 37°C and 4% CO₂. The radiolabeled cells were washed 3 times with 4 mL of cold 10% FCS RPMI-1640 medium and were then diluted with 10 mL of medium to achieve a final concentration of 1×10^5 cells·mL⁻¹. The labeled cells were kept on ice until the assay was performed.

Triplicates of 100 µL of PBMC at concentrations of 2×10^6 , 1×10^6 , and 0.5×10^6 cells·mL⁻¹ were mixed with a 100 µL suspension of radiolabeled (⁵¹Cr) target cells at 1×10^5 cells·mL⁻¹ and were then centrifuged (1 min at 37 °C and 160 *g*) in a 96-well round bottom microtiter plate (Sarstedt, St. Leonard, Quebec, Canada). After

incubation for 4 h at 37 °C, 4% CO₂, the cell mixture was centrifuged for a further 5 min at 4 °C and 225 g. One hundred microliters of supernatant were then withdrawn and transferred to polystyrene, round bottom tubes (12 x 75 mm) (Falcon, Lincoln Park, NJ, USA). The radioactivity of the supernatant was determined by a Cobra Automated Gamma Counter (Model 5002, Packard Instruments, Downers Grove, Illinois, USA). Spontaneous release of ⁵¹Cr was assessed by incubating 100 µL of medium with 100 µL of target cells. The maximum potential release of radioactive material was determined by incubating 100 µL of 1 % Triton X-100 (a biodegradable non-ionic surfactant) (Sigma, St. Louis, MO, USA) with 100 µL of target cells. The percentage of ⁵¹Cr release (cytolytic activity) was calculated using the formula: percentage lysis = [(test - spontaneous) cpm/(maximum - spontaneous) cpm] x 100 %. The exponential curve fitting method of Pross *et al.* (77) was used to determine lytic units from the values obtained for percent lysis at three different effector-target ratios (20:1, 10:1 and 5:1). The lytic unit was defined as the number of effector cells required to lyse 20% of 10,000 target cells. Results were expressed as the number of lytic units contained in 1 x 10⁶ PBMC (LU·10⁶ PBMC⁻¹) (77). Lytic units were also calculated on a per NK cell basis, using the formula: single cell cytolytic activity = lytic units/[% NK cells x (10⁶ PBMC - monocytes)].

Biochemical analysis. K₃-EDTA and EGTA/reduced glutathione-treated vacutainers were mixed by gentle inversion, placed in an ice water bath for no more than 30 min, and then were centrifuged for 15 min at 4°C and 2250 g. The plasma samples were separated from the packed red cells, transferred to Eppendorf tubes and immediately frozen and stored at -70°C. Plasma concentrations of interleukin-6 (IL-6)

were determined using an enzyme immunoassay kit (R & D Systems, Minneapolis, Minnesota, USA). Total plasma concentrations of cortisol were determined by radioimmunoassay, using standard commercially available kits (Diagnostic Products, Los Angeles, CA). Plasma catecholamine concentrations were measured using a gas chromatography-mass spectrometry (GC-MS) system (118); values are reported as unbound norepinephrine (NE) and epinephrine (E) concentrations.

Statistical Analysis. Results are expressed throughout as means \pm S.E. The statistical significance of changes in physiological, immunological, and hormonal parameters was analyzed using one- and two-way (trial by time) repeated measures analyses of variance. Comparison between the control condition and 38°C water immersion provided information on the effects of heat and subsequent response to cold exposure. Comparisons between the control condition versus exercise in cold water and passive heating versus exercise in 35°C water provided information on the effects of exercise and subsequent response to cold exposure. Lastly, comparisons between exercise pre-treatments allowed for the analysis of the influence of an exercise-induced rise in core temperature. Specific post-hoc contrasts were used to explore significant main effects and interactions. Bonferroni adjustments were made for multiple comparisons, a probability (P) value of < 0.001 being accepted as statistically significant.

From the data obtained during cold exposure, correlations between cell counts, heart rate, rectal temperature and hormone levels were determined using forward, stepwise multiple regression analysis. Each cell type was analyzed as the dependent variable with the other variables (heart rate, rectal temperature and hormonal levels) set as independent variables. In a separate analysis, IL-6 was analyzed as the dependent

variable with 4 independent variables (rectal temperature, cortisol, nor-epinephrine and epinephrine). The forwards stepwise multiple regression analysis sequentially determines non-significant variables which should be excluded in a predictive equation. Variables are progressively eliminated from the analysis and a reduced equation is recalculated using the remaining variables (95). These analyses were completed in order to gain an understanding of the mechanisms that may be involved in some of the changes observed. For each regression analysis, a probability (P) value of < 0.05 was accepted as statistically significant for individual terms in the equation. All statistical calculations were performed using StatViewTM and SuperANOVATM microcomputer software packages (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Physiological responses. A slight, although statistically significant, 0.3°C reduction in rectal temperature occurred when subjects sat in water at 35°C (Figure 13). In contrast, rectal temperatures were significantly increased 0.7°C within 45 min of passive heating. Rectal temperatures were also significantly increased 0.6°C within 30 min of exercise in 35°C water and were not significantly altered when subjects exercised in 18°C water (hereafter described as exercise with a thermal clamp). For each of the pre-treatment exercise conditions, subjects exercised on a modified underwater cycle ergometer at an average of 55 % of their personal $\dot{V}\text{O}_{2\text{peak}}$.

Prior to entry into the cold chamber, rectal temperatures returned to pre-treatment baseline levels following control condition (sitting in 35°C water) as well as following exercise with a thermal clamp. Rectal temperature was some 1°C higher following the

pre-treatments of passive heating or exercise in 35°C water (Figure 13). Following pre-treatment with exercise with a thermal clamp, rectal temperatures were significantly reduced within 15 min of cold air exposure. Seventy-five minutes of cold air exposure significantly reduced rectal temperatures not only in the control condition but also when subjects had been pre-treated by exercise in water at 35°C. After 1 h and 45 min of cold exposure, rectal temperatures were significantly reduced irrespective of the pre-treatment protocol. Rectal temperature reached its lowest value when subjects had exercised with a thermal clamp prior to the cold air exposure.

Heart rates rose during passive heating [pre = 76 (2) beats·min⁻¹; post = 100 (4) beats·min⁻¹] and were significantly elevated in response to exercise with and without a thermal clamp [exercise in water at 18 °C: pre = 77 (5) beats·min⁻¹ vs post = 123 (5) beats·min⁻¹ and exercise in water at 35 °C: pre = 84 (3) beats·min⁻¹ vs post = 150 (6) beats·min⁻¹]. Heart rates returned to baseline values immediately prior to cold exposure, irrespective of pre-treatment.

Total leukocytes and differentials. Prior to each treatment, the average leukocyte count (Figure 14) was within the expected normal range of 4.5 - 11.0 x 10⁹ cells·L⁻¹ (38). Cell counts were not significantly altered when the subjects remained seated in the 35°C and 38°C water baths. In contrast, exercise with or without a thermal clamp induced a significant rise in leukocyte, granulocyte and lymphocyte counts.

At entry into the cold chamber, total leukocyte counts were significantly higher when subjects had previously exercised with a thermal clamp. After 1 hour of cold exposure, total white cell counts had increased significantly in all four pre-treatment

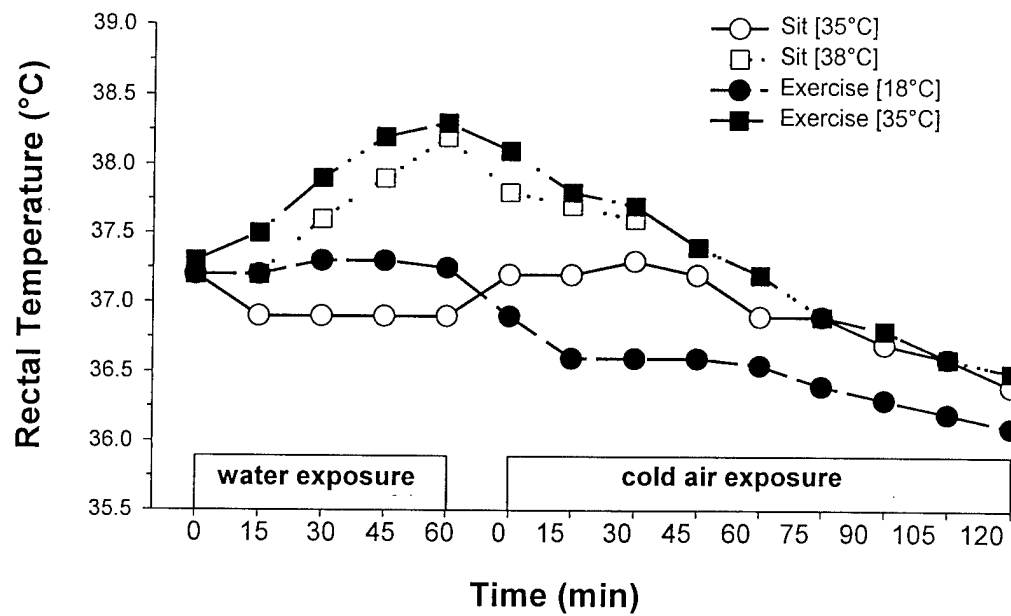


Fig. 13. Rectal temperature during water exposure and cold air exposure.

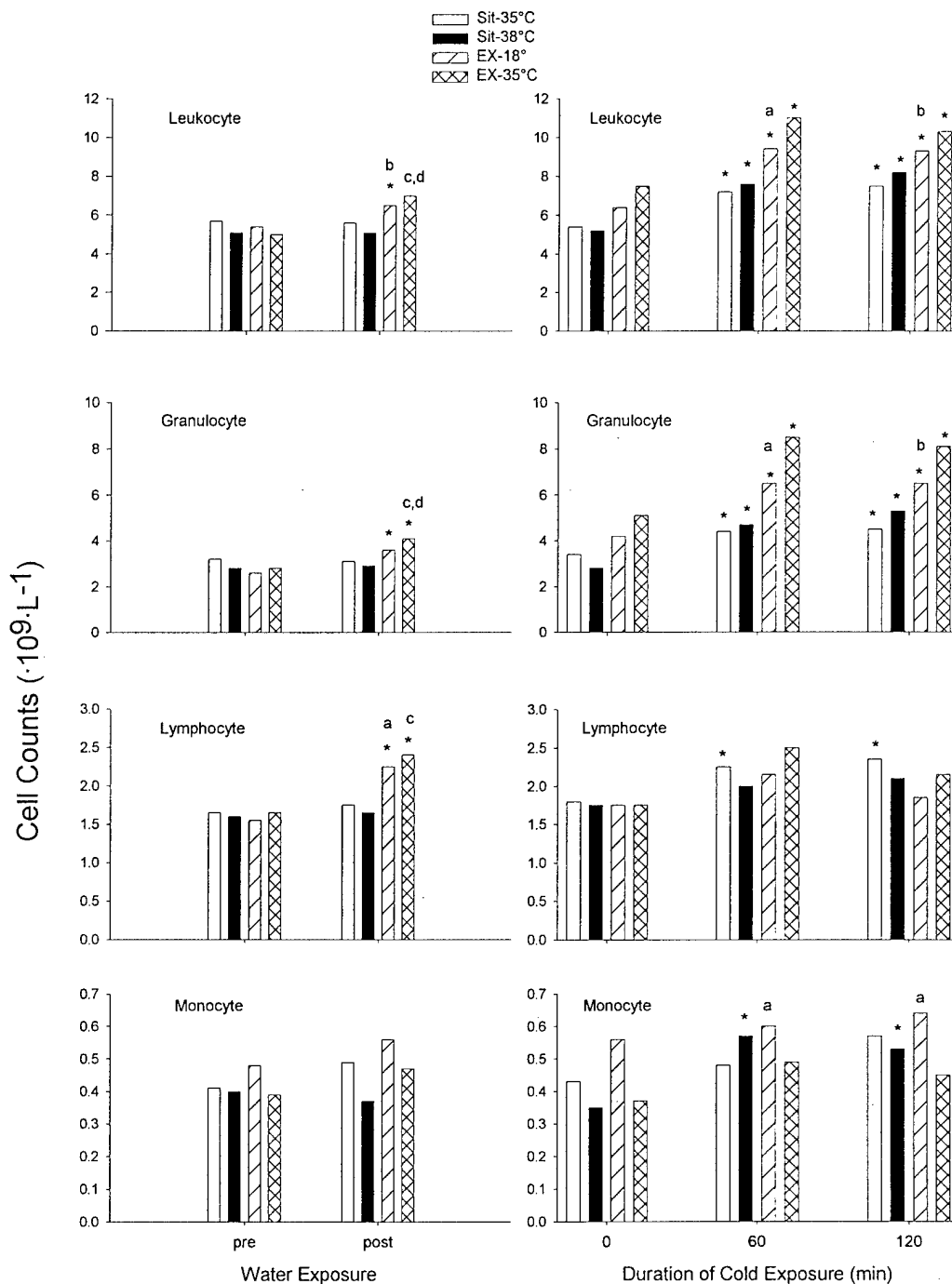


Fig 14. Leukocyte, granulocyte, lymphocyte, and monocyte counts during water exposure and cold air exposure. *, Significant difference ($P < 0.05$) from initial measurement (pre vs. post; time 0 vs. time 60 & 120); a, Sit-35°C different than EX-18°C; b, Sit-35°C different than EX-35°C; c, Sit-38°C different than EX-35°C; d, EX-35°C different than EX-18°C at corresponding time.

conditions and values remained elevated at 2 h of cold exposure. Significantly more leukocytes were recruited into the circulation after 1 h of cold exposure, if the pre-treatment was exercise with a thermal clamp rather than the control condition.

Granulocyte counts followed a similar pattern of response to the total leukocyte counts, increasing significantly within 1 h of cold air exposure (Figure 14). Compared to the control condition, a significantly greater number of granulocytes were recruited into the peripheral circulation when subjects had previously exercised with a thermal clamp for 1 h. Similarly, when compared to the passive heating pre-treatment condition, granulocyte counts tended to be higher if subjects had previously exercised in 35°C water, but this was not statistically significant.

Lymphocyte counts were significantly increased by cold air exposure if this was preceded by the control pre-treatment. Lymphocyte counts also tended to be elevated in response to cold exposure in the other three pre-treatment conditions, but the changes were no longer statistically significant. After pre-treatment with exercise (with or without a thermal clamp), lymphocyte counts reached peak levels within 1 h of cold air exposure.

Monocyte counts were not significantly altered by any of the pre-treatment conditions. Within 1 h of cold air exposure, the monocyte count was significantly increased when subjects had previously sat for 1 h in a warm water bath. Recruitment of monocytes during cold exposure was also significantly greater with pre-treatment by exercise with a thermal clamp than with the control pre-treatment.

Forwards stepwise multiple regression analyses were performed in order to gain an understanding of the possible mechanisms contributing to the cellular changes observed during cold exposure. The variables initially included in the model were heart

rate, rectal temperature, cortisol, norepinephrine and epinephrine; the analyses indicated that both leukocyte and granulocyte counts were most closely related to heart rate and norepinephrine concentrations (Table 2). Lymphocyte counts were most closely related to norepinephrine. With the exception of monocytes, rectal temperature had no influence on circulating leukocyte subsets.

Lymphocyte subsets. None of the four pre-treatments had any effect on the circulating pan T (CD3⁺), T-helper (CD4⁺) and B (CD19⁺) cell counts (data not shown). In contrast, exercise with and without a thermal clamp recruited significant numbers of cytotoxic/suppressor T-cells (CD8⁺) [exercise in water at 18°C: pre = 0.475 cells·10⁹·L⁻¹ (0.062) cells·10⁹·L⁻¹ vs post = 0.711 (0.038) cells·10⁹·L⁻¹ and exercise in water at 35°C: pre = 0.460 (0.070) cells·10⁹·L⁻¹ vs post = 0.765 (0.082) cells·10⁹·L⁻¹] and NK-cells (CD3⁺/CD16⁺56⁺) into the circulation (Figure 15).

Following the control pre-treatment, 2 h of cold exposure significantly increased levels of circulating T (CD3⁺) and B (CD19⁺) cells [pre-cold exposure CD3⁺ cell counts, time 0 = 1.18 (0.10) cells·10⁹·L⁻¹ blood vs post cold exposure CD 3⁺ cell counts, time 120 min = 1.36 (0.08) cells·10⁹·L⁻¹; pre-cold exposure CD19⁺ cell counts, time 0 = 0.17 (0.03) cells·10⁹·L⁻¹ vs post cold exposure CD 19⁺ cell counts, time 120 min = 0.22 (0.03) cells·10⁹·L⁻¹]. NK (CD3⁺/CD16⁺56⁺) cell counts increased significantly in response to cold exposure following both the control pre-treatment and exercise in 35°C water. Pre-treatment with either passive heating or exercise with a thermal clamp resulted in slightly higher baseline NK cell counts; when cold exposure followed either of

Table 2. Multiple regression analysis for prediction of leukocyte subset and lymphocyte counts by heart rate, rectal temperature and hormone concentrations.

Variable	R ²	Heart Rate	Rectal Temperature	Cortisol	Nor- Epinephrine	Epinephrine
Total leukocytes	0.446	0.0018	ns	ns	0.0012	ns
Granulocytes	0.397	0.0042	ns	ns	0.0078	ns
Monocytes	0.161	ns	0.0372	ns	ns	ns
Lymphocytes	0.273	ns	ns	ns	0.0005	ns
CD3 ⁺	0.201	ns	0.0348	ns	0.0057	ns
CD4 ⁺	0.015	ns	ns	ns	ns	ns
CD8 ⁺	0.216	ns	ns	ns	0.0071	ns
CD19 ⁺	0.423	0.0483	ns	ns	<0.0001	0.0139
CD3 ⁻ ,CD16 ⁺ 56 ⁺	0.192	ns	ns	ns	ns	ns

Values are shown in terms of individual probability. NS, not significant

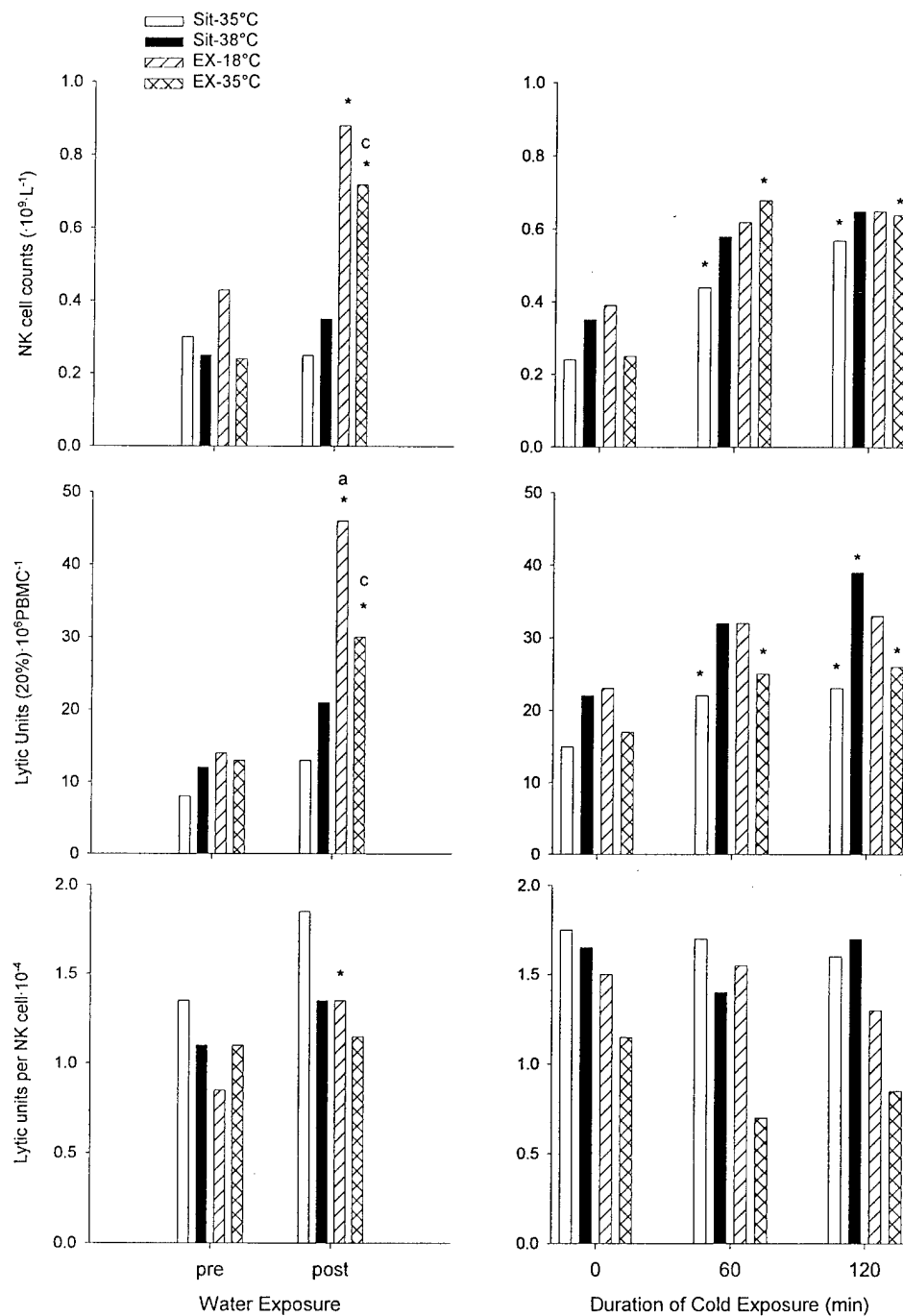


Fig 15. Natural killer cell counts, total natural killer cell activity (lytic units) and lytic units per number of natural killer cells during water exposure and cold air exposure. *, Significant difference ($P < 0.05$) from initial measurement (pre vs. post; time 0 vs. time 60 & 120); a, Sit-35°C different than EX-18°C; c, Sit-38°C different than EX-35°C at corresponding time.

these two conditions, NK cell counts again tended to increase, but the change no longer reached statistical significance.

Stepwise multiple regression analyses explained from 2 to 42 percent of total variance, depending on which lymphocyte subset was examined (Table 2). With the exception of CD3⁺ cells, rectal temperature had no influence on circulating lymphocyte subsets. Epinephrine was an important determinant of CD19⁺ cells, but norepinephrine accounted for much of the variance in CD3⁺, CD8⁺ and CD19⁺ cell counts. None of the variables examined could significantly account for changes in NK cell counts.

NK cell activity. Total NK cell activity measured in lytic units (Lytic Units $\cdot 10^6$ PBMC⁻¹) increased significantly in response to exercise with and without a thermal clamp. Passive heating and sitting in 35°C water did not significantly alter total NK cell activity. Pre-treatment with the control condition as well as with exercise with a thermal clamp resulted in significantly higher levels of total NK cell activity when subjects entered the cold chamber.

Total NK cell activity increased significantly within 60 min of cold exposure for the control pre-treatment and in response to prior exercise without a thermal clamp. Passive heating followed by 2 h of cold exposure also induced a significant rise in total NK cell activity. Cold exposure following exercise with a thermal clamp tended to increase total NK cell activity, although levels did not reach statistical significance. A stepwise regression analysis (which began by correlating NK cell number, rectal temperature, cortisol, epinephrine and norepinephrine with NK cell activity) indicated that NK cell activity was most closely related to changes in circulating NK cell counts (multiple $R^2 = 0.337$, $p < 0.0001$).

When total NK cell activity was adjusted on a per cell basis relative to NK cell counts, lytic activity per cell was significantly increased in response to exercise with a thermal clamp and remained elevated at entry into the climatic chamber. However, there were no longer any significant differences between pre-treatment conditions in response to cold exposure.

Plasma hormone levels. The responses of the different stress hormones (free nor-epinephrine, free epinephrine and cortisol) to the various treatments are presented in Figure 16. Cold exposure preceded by either the control pre-treatment or passive heating induced a significant rise in plasma nor-epinephrine concentration within 60 min (Figure 16). When subjects exercised with a thermal clamp prior to cold exposure, plasma nor-epinephrine levels increased significantly only after 120 min. A similar pattern of response occurred when subjects exercised in 35°C water before entering the cold chamber; however, in part because of greater variability in the data, the response was no longer statistically significant. Cold exposure following the control pre-treatment did not significantly alter levels of circulating epinephrine. When preceded by passive heating, plasma epinephrine concentration was significantly higher after two hours of cold exposure.

With the exception of the condition involving pre-treatment with passive heating, cold exposure had no significant effect on plasma cortisol levels. For this condition only, 1 h of cold exposure led to significant reductions in plasma cortisol levels.

IL-6 levels. Passive heating and exercise with a thermal clamp did not significantly alter plasma levels of IL-6 (Figure 17). However, exercise without a thermal clamp induced a significant elevation in IL-6. Prior to entry into the cold chamber, levels

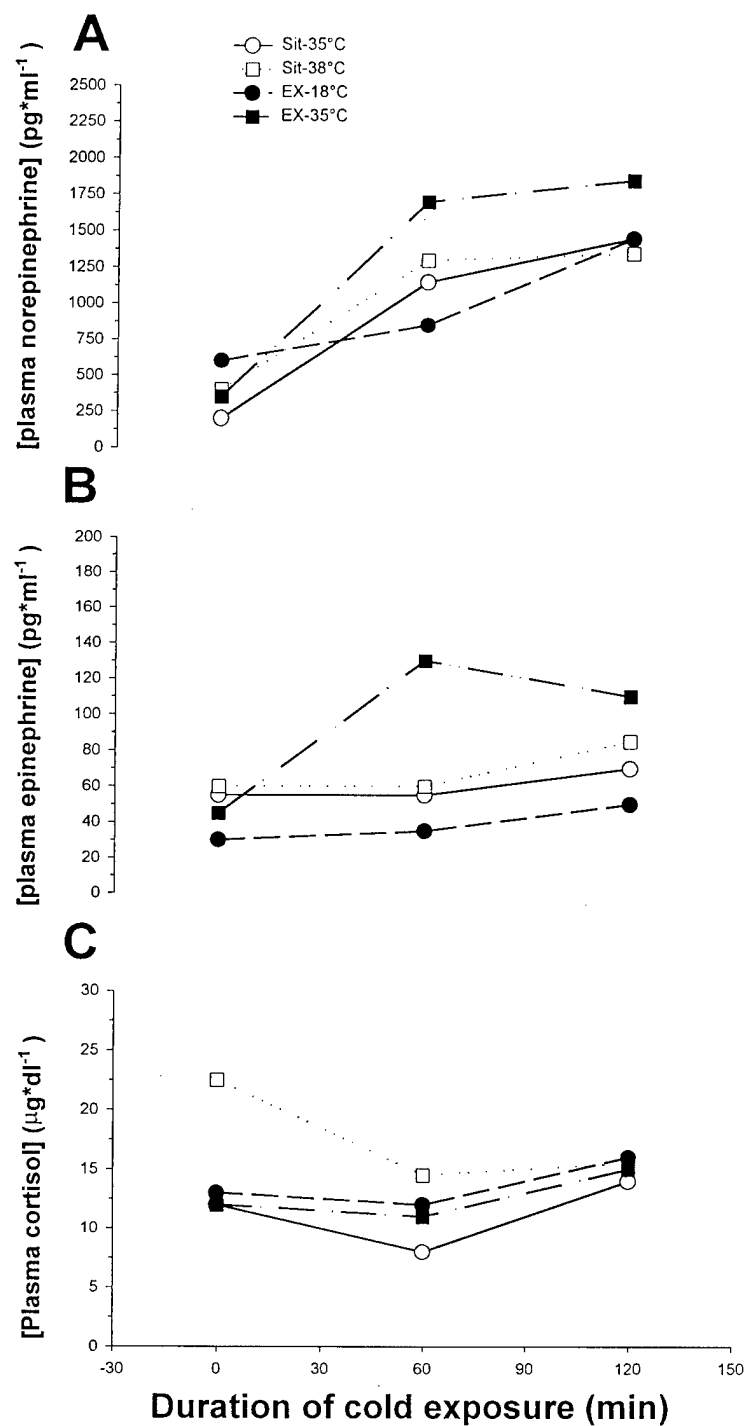


Fig. 16. Plasma norepinephrine (A), epinephrine (B), and cortisol (C) concentrations during cold air exposure following each treatment.

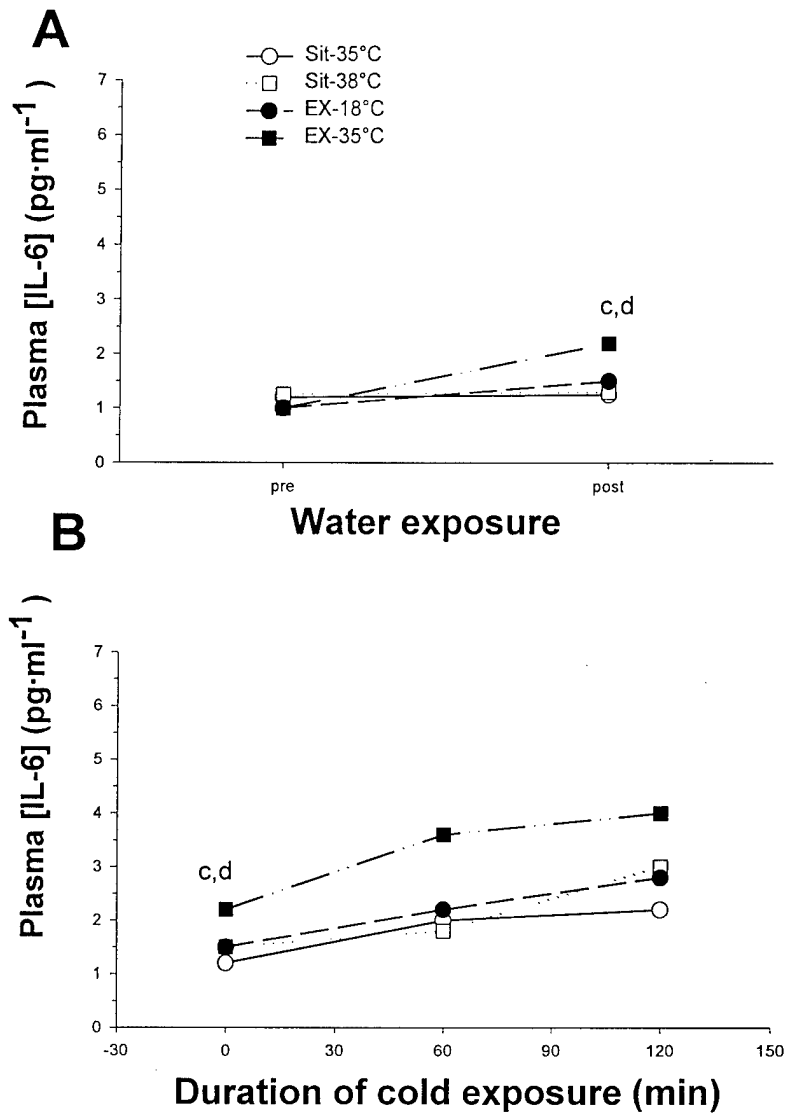


Fig. 17. Interleukin-6 concentrations during water exposure (A) and cold air exposure (B) during each treatment. c, Sit-38°C different ($P < 0.05$) than EX-35°C; d, EX-35°C different ($P < 0.05$) than EX-18°C at corresponding time.

of IL-6 were significantly higher compared to baseline in each of the exercise pre-treatments.

In the control condition, one hour of cold exposure induced a significant rise in plasma IL-6 concentration. When cold exposure was preceded by either passive heating or exercise with a thermal clamp, plasma IL-6 levels were significantly elevated after 2 h. Prior exercise without a thermal clamp also tended to induce an elevation in response to cold exposure, but these results did not reach levels of significance. A stepwise multiple regression analysis began by correlating IL-6 with rectal temperature, cortisol, nor-epinephrine and epinephrine concentrations. Two variables (cortisol and norepinephrine) were retained in the equation with a multiple R^2 of 0.191, $p = 0.0015$ for the equation as a whole.

DISCUSSION

The present study examined the impact of cold exposure on selected aspects of immune function, together with the possible modulating effects of prior passive heating and moderate exercise (with or without a thermal clamp). During cold exposure, the body attempts to maintain a normal body temperature by increasing heat production and minimizing heat loss (81). This is accomplished through involuntary tonic muscular activity, rhythmic muscular activity (shivering), peripheral vasoconstriction and the suppression of sweat secretion. Although primarily thermoregulatory, these mechanisms can also induce metabolic and hormonal changes which in turn alter immune status.

Anecdotal and experimental reports have suggested that cold exposure may be associated with an increased risk of infection (91). Laboratory animal research involving

prolonged cold exposure or cold water immersion, demonstrated a reduction in the function of certain immune parameters (i.e., lymphocyte proliferation; NK cell activity) (1,43,92,112). Such extreme physiologic conditions do not represent the typical conditions which humans may encounter during normal daily athletic activities or military operations. Therefore, this study was designed to examine the acute effects of a brief cold exposure. The parameters evaluated were those components of the immune system which we hypothesized would be most responsive to such a challenge. NK cell activity was included since NK cells represent one of the first lines of defense against virally infected cells, tumor cells and certain micro-organisms. Our findings that the selected aspects of immune function measured in this study were not adversely affected by a moderate exposure to cold air is reassuring. Indeed, we have demonstrated that exposing subjects to a cold environmental chamber (5°C) for 2 hours can be immunoenhancing and that moderate exercise (with a thermal clamp) can further augment the response of certain parameters to subsequent cold exposure.

Effects of passive heating and moderate exercise (with and without a thermal clamp)

Cellular Responses. In confirmation of our previous work (87), we did not observe any increase in either total or differential leukocyte cell counts when our subjects were passively heated. Passive heating increased core body temperature by only 1°C and a greater rise in body temperature (at least 2°C) seems necessary to induce significant changes in circulating cell counts. Both Kappel et al.,(56) and Downing et al. (28) immersed their subjects in a hot water bath (39.5-40°C) for at least 2 h in order to raise

core temperature (2.0 - 2.5°C); significant increases in total and differential leukocyte counts were then observed.

Our observation that total leukocyte, granulocyte and lymphocyte counts rise in response to underwater cycle ergometer exercise is consistent with the findings reported by other investigators in our laboratory (23,82). Similarly, thermal clamping attenuated this leukocyte and granulocyte response. A combination of a relatively small sample size, inter-subject variability, a moderate exercise intensity (55% $\dot{V}O_{2peak}$) and a mild 1°C rise in rectal temperature may explain why we did not observe any differences in lymphocyte and monocyte responses between the different exercise conditions. Increases in cardiac output (via shear stress) and/or changes in levels of circulating hormones (leading to alterations in adhesion molecules and recruitment of cells from reservoirs) account for the exercise-induced changes in cell counts (67). Thermal clamping by cold water immersion not only attenuates the hormonal responses, but also blocks the additional increase in cardiac output that would otherwise have been induced by cutaneous dilatation. Thus, we had expected that the leukocyte response to exercise would be attenuated by the thermal clamp.

In the present study, none of the four water pre-treatments significantly altered CD3⁺, CD4⁺ or CD19⁺ cell counts during subsequent exercise. In contrast, NK cells and cytotoxic T- lymphocytes (CD8⁺) and were significantly increased in response to exercise (with or without a thermal clamp). Cytotoxic cells (primarily NK cells) have the greatest number of β_2 adrenergic receptors (59,63) and tend to be more responsive to the release of catecholamines than other lymphocyte subsets (40). In general, the rise in circulating NK cell count was adequate to explain the parallel rise in NK cell activity observed in

response to exercise. However, NK cell activity per cell was significantly increased in response to exercise in cold water, indicating that other humoral factors may have contributed to the enhanced activity observed in this condition.

IL-6 response. Of the various pro-inflammatory cytokines (IL-1, TNF and IL-6) we chose to analyze plasma IL-6. IL-6 is one of the few cytokines that is consistently responsive to exercise. The response of other pro-inflammatory cytokines is less consistent and indeed these cytokines are difficult to detect in plasma by currently available techniques. The finding that plasma IL-6 concentration was significantly increased in response to exercise without a thermal clamp is consistent with other studies of prolonged and strenuous exercise (3,29,39,70).

Immune changes during cold exposure: effects of prior heating and exercise

Cellular responses. In accord with the results of both animal (101) and human (53) studies, acute cold stress increased circulating leukocyte and neutrophil counts. Jansky et al. (53) reported increases in both red cell and leukocyte counts when their subjects were initially immersed in cold (14°C) water for 1 h. However, this response seems only transient, as chronic cold-water immersion (3 times a week for 6 weeks), did not alter the resting leukocyte or granulocyte counts. The cold-induced leukocytosis was significantly augmented by prior exercise with a thermal clamp. Perhaps this is because increased leukocyte counts tend to persist following exercise, giving an additive cellular response to cold plus prior exercise.

When preceded by the control condition, cold exposure significantly increased levels of circulating T- (CD3⁺) and B- (CD19⁺) cells. This cellular response was no

longer significant in the face of pre-treatment with passive heating or exercise (with or without a thermal clamp). These results may partly be explained by the increase in subject variability that occurred in response to pre-treatment. However, a lymphocytopenia has also been reported following exercise (75), and this may have attenuated the rise in T- and B- cells that would otherwise have occurred in the cold. Without assessment of the proliferative response of these cell subtypes to mitogens, the biological significance of such minor changes remains questionable.

T-helper (CD4⁺) and T-cytotoxic/suppressor (CD8⁺) cell counts were not significantly altered by 2 h of cold exposure (5 °C). Similarly, 1 h of cold (14 °C) water immersion did not significantly alter circulating concentrations of these cells (53). In contrast, Hennig and associates (46) reported a decrease in peripheral CD4⁺ counts when body temperature was reduced (0.25 °C) by cold exposure (5 °C for 20 min) as well as by administration of a 5HT_{1A} agonist. For the group receiving the 5HT_{1A} agonist, cortisol was demonstrated to be the mediator of changes in CD4⁺ cells. However, for the group exposed to cold air, the reduction of CD4⁺ cells showed no relationship to changes in cortisol concentration. The discrepant results may be due to the timing of samples. Since our first data were collected 1 h after cold exposure, there could have been an undetected reduction in cell counts during the first 20 min of cold exposure, associated with an early "stress" response, with a return to baseline levels after 1 h of cold exposure. Nevertheless, this is not very likely, since the "stress" of cold exposure tends to be cumulative, and related to the fall in core temperature.

Animal studies have demonstrated either a decrease (1,54,112) or no change (92) in NK cell number and activity in response to cold exposure. In these studies, mice

and/or rats were subjected to repeated bouts (2 - 5 min) of very cold (4°C) water stress over several days (4 - 5 days) (1,54,92) as well as to prolonged cold-air exposure (4°C) for up to 16 days (112). Such conditions can be stressful for the animal. It remains unclear how much of this response is attributable to a generalized "stress" response rather than cold exposure, and how far reactions are affected by acclimatization to cold. Most of these studies have postulated that a "stress" - induced release of corticosteroids accounts for the reduction in NK cell number and activity that occurs with cold exposure.

In contrast, studies involving human subjects have demonstrated an increase in NK cell activity in response to local or generalized cold exposure (26). Delahanty et al. (26) examined the effects of a cold-pressor task on NK cell function. In their study, 10 male subjects (aged 20 - 45 yr) were required to place their hands intermittently in very cold (3°C) water for a total submersion time of 3 min (the durations of immersion were 30, 20, 45, 15, 40, and 30 sec with a 30 sec rest period in-between each submersion). Here, there was a local stressful stimulus, with little change of core temperature. They demonstrated a trend toward an increase in NK cell activity 2 min into the cold-pressor task. Lackovic et al. (60) exposed 8 naked male volunteers (20 - 26 yr) to a cold (4°C) room for 30 min. Body temperatures showed only a moderate decrease (an average of 0.45°C), but NK cell activity was significantly increased. Similarly, our study has demonstrated that NK cell counts and activity increased in response to cold exposure which caused quite modest reductions in core temperatures (ranging between 0.6°C and 1.6°C).

IL-6 response. Our finding that plasma concentrations of IL-6 rose in response to cold exposure is in contrast to the work of Jansky et al. (53). They reported a slight and

non-significant trend to a reduction in the concentration of IL-6 when subjects were immersed for 1 h in cold (14°C) water. They also showed that repeated cold water immersions over a period of three to six weeks had no significant effect on resting levels of circulating IL-6. It is possible that their cold stimulus was not sufficiently prolonged or severe to induce any significant changes. We observed a significant rise in plasma levels of IL-6 when cold exposure was preceded by the control condition, passive heating or exercise with a thermal clamp, but not in the condition when initial core body temperature was highest (exercise in water at 35°C).

Several physical stressors increase circulating levels of IL-6 (71,75,119); however, the mechanisms involved have not been clearly identified. Pedersen et al. (75) have proposed that muscle damage associated with eccentric muscular activity induces the release of pro-inflammatory cytokines. Zhu et al. (120) provided experimental data showing that the secretion of IL-6 from mouse peritoneal macrophages after cold water stress (5 min swim tests in 10°C water) may be related to increased tissue levels of immunoreactive substance P. In our study, regression analyses indicated that the changes in levels of IL-6 were most closely related to plasma levels of norepinephrine and cortisol. Animal studies have shown that catecholamines stimulate endogenous IL-6 secretion, whereas glucocorticoids inhibit it (73). It is possible that norepinephrine exerts its effects by a cyclic AMP-dependent mechanism, since cyclic AMP can modulate cytokine production. Szabo et al. (102) have demonstrated that pre-treatment with isoproterenol increased the LPS-induced production of IL-6 in rats.

Hormonal responses. Catecholamines and cortisol play a major role in the physiological responses to cold exposure. Norepinephrine mediates "futile" metabolic

cycling in white and brown adipose tissue, together with the acute, general cutaneous vasoconstriction that occurs in the extremities (61). Epinephrine and cortisol facilitate the metabolism of glucose and triglycerides, also contributing to increased heat production.

Levels of plasma norepinephrine were significantly increased within 1 h of cold-air exposure in the control condition as well as following passive heating and exercise with a thermal clamp. Presumably because of higher initial core temperatures, changes were not statistically significant following pre-treatment with exercise at 35°C. Peripheral vasoconstriction, induced by sympathetic release of nor-epinephrine, is one of the first responses to cold air exposure (61). Thus, it is not surprising that a significant rise in plasma norepinephrine concentration was observed. In contrast, (as reported by others, 24), cold exposure did little to alter levels of circulating epinephrine and cortisol. Although cortisol and the catecholamines (epinephrine and norepinephrine) have typically been referred to as "stress" hormones, the release of cortisol tends to lag behind that of catecholamines. This may explain why we did not observe a cortisol response to cold exposure. A 24-hour urine sample would have provided a more complete picture of cortisol responses.

Norepinephrine can mobilize cells through adrenergic receptor stimulation (40) as well as by its action on sympathetic nerve terminals within the lymph nodes and spleen (5,13). Cortisol stimulates the release of granulocytes from the bone marrow and reduces lymphocyte counts by inhibiting their entry into, and facilitating their egress from the circulation (24). It is unlikely that changes in the levels of circulating cortisol contributed to a delayed neutrophilia or lymphopenia in this particular design, as circulating cortisol levels tended to remain the same during cold exposure. Our multiple regression analyses

suggest rather that the rise in circulating leukocytes during cold exposure may be attributed to a norepinephrine mediated mobilization of demarginated cells. These results should be interpreted with caution, since correlation coefficients were relatively low. Nevertheless, our results support the hypothesis that the observed changes in cell counts may be due to a change in levels of circulating hormones induced by a combination of exercise and changes in core body temperature.

Further work should examine if other components of the innate immune system (neutrophil oxidative burst) and components of the adaptive immune system (lymphocyte proliferative responses and immunoglobulin levels) respond in a similar manner. Research should also be completed to examine the influence of more prolonged and repeated bouts of cold exposure on immune function. Adverse effects might possibly be observed if the combination of cold exposure and prior exercise were sufficient to deplete key nutrients important to cellular immune responses. Finally, investigators should explore whether the rate of any adaptations to a cold environment is modified when bouts of physical activity are performed immediately before or during cold exposure.

This study suggests that despite popular beliefs that cold exposure can precipitate a viral infection, the innate component of the immune system is not adversely affected by a brief period of cold exposure. Indeed, the opposite seems the case. The fall in core body temperature resulting from cold exposure led to a consistent and statistically significant mobilization of circulating cells, an increase in NK cell activity and elevations in circulating IL-6 concentrations. Moreover, in agreement with one of our hypotheses, prior exercise with a thermal clamp significantly augmented the leukocyte, granulocyte and monocyte response to cold exposure. Prior passive heating and exercise without a

thermal clamp also tended to augment the effect of cold exposure alone, but due to the small sample size and intersubject variability, these changes were not statistically significant.

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